Universidade de Aveiro Departamento de Biologia
2016

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Predisposição de *Eucalyptus* a *Neofusicoccum kwambonambiense* causada por *stress* hídrico

Eucalyptus predisposition to *Neofusicoccum kwambonambiense* under water stress

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Molecular e Celular, realizada sob a orientação científica do Doutor Artur Jorge da Costa Peixoto Alves, investigador principal do Departamento de Biologia da Universidade de Aveiro e co-orientação da Doutora Glória Catarina Cintra da Costa Pinto, professora auxiliar convidada do Departamento de Biologia da Universidade de Aveiro.

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To curiosity, the spark behind science

o júri

presidente	Prof. Doutora Maria Paula Polónia Gonçalves Professora Associada do Departamento de Biologia da Universidade de Aveiro
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orientador	Prof. Doutor Artur Jorge da Costa Peixoto Alves Investigador Principal do Departamento de Biologia e CESAM da Universidade de Aveiro

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palavras-chave

Floresta, Desempenho das plantas, *Eucalyptus globulus*, Interações bióticas e abióticas, Interações planta-patogénio

resumo

Em Portugal, o eucalipto, particularmente o *Eucalyptus globulus*, ocupa mais de 800 000 ha. Devido a ser uma importante fonte de biomassa para painéis de fibras, carvão industrial, lenha e pasta de papel, tornou-se um género chave de considerável importância económica. Contudo, a produtividade de *E. globulus* tem encontrado novas pressões, sendo a seca resultante das alterações climáticas, uma das mais hostis. A seca pode levar a uma diminuição do crescimento e produtividade: diretamente; ou indiretamente através do aumento da suscetibilidade a agentes patogénicos através da predisposição. O fungo ascomiceto *Neofusicoccum kwambonambiense* é um agente fitopatogénico endofítico oportunista que se sabe afetar severamente *E. globulus*, e cuja presença já fora confirmada em Portugal. Tomando tal em consideração, torna-se importante avaliar o efeito de predisposição que a seca poderá ter na interação *N. kwambonambiense - E. globulus.* Para tal foram criados quatro grupos de tratamento: *E. globulus* foram primeiramente sujeitos a um período de aclimatização de 66 dias no qual foram periodicamente irrigados (80% de capacidade de campo). Seguidamente, dois grupos foram sujeitos a uma diminuição progressiva da irrigação. Os outros dois grupos permaneceram bem regados. Uma vez que os tratamentos stressados atingiram 18% de capacidade de campo (23 dias), um grupo bem regado e um grupo stressado foram inoculados com *N. kwambonambiense*. Todas os tratamentos foram mantidos nestas condições durante um período de 66 dias, findo o qual foi obtido um conjunto de parâmetros morfológicos, fisiológicos e bioquímicos. As plantas bem regadas, apesar de terem sido inoculadas com *N. kwambonambiense* apresentaram um aumento dos parâmetros fotossintéticos o que terá permitido a defesa da planta através de uma produção amplificada de açúcares, prolina e ácido salicílico. Danos oxidativos (parcialmente observados através do conteúdo em *malondialdeído*) foram evitados, em parte, devido à ação da prolina e açúcares solúveis. O *stress* hídrico levou a uma diminuição do crescimento confirmado pela redução do conteúdo em ácido-indole-acético. Ocorreu uma diminuição do potencial hídrico, a qual, em conjunto com o aumento do ácido abscísico, levou ao fecho dos estomas e diminuição da fotossíntese. Os danos oxidativos não foram controlados, afetando o estado do *E. globulus*. Ademais, o *N. kwambonambiense* provocou um aumento do conteúdo em ácido jasmónico, típico de agentes patogénicos necrotróficos, o que poderá sugerir que o fungo passou de um estilo de vida hemibiotrófico para necrotrófico, à medida que as células degeneravam. Os *E. globulus* stressados apresentavam maiores lesões externas e cancros, conjuntamente com uma maior progressão interna do fungo. Os nossos resultados comprovam que a seca criou um melhor substrato para o desenvolvimento do fungo e diminuiu a capacidade de resposta da planta. Tal resultou num aumento da suscetibilidade e severidade da doença confirmando a predisposição.

keywords

Forest trees, Plant performance, *Eucalyptus globulus*, Biotic and abiotic interactions, Plant-pathogen interactions

abstract

In Portugal, *Eucalyptus*, particularly *Eucalyptus globulus*, occupies more than 800 000 ha and, due to being a major source of biomass for fiberboard, industrial charcoal, fuel wood and paper pulp, has become a key genus, with a considerable economic importance. However, *E. globulus* productivity faces new pressures, with climate change-driven drought as one of the most hostile ones. Drought can lead to growth impairment and yield reduction: directly; or indirectly, through the increase of plant susceptibility to pathogens by a predisposition mechanism. *Neofusicoccum kwambonambiense* is an endophytic opportunist phytopathogen known to severely affect *E. globulus*, whose incidence has already been reported in Portugal. Taking all in consideration, it is of major importance to assess the predisposition effect that drought may have on the *N. kwambonambiense* - *E. globulus* interaction. For such purpose, four treatment groups were established: *E. globulus* were firstly subjected to a 66-days acclimation period in which plants were periodically watered (80% of field capacity). After that, two groups were exposed to a progressive water supply restriction. The other two remained well-watered. Once water-stressed plants achieved 18% of field capacity (23 days), a well-watered and a water-stress group were inoculated with *N. kwambonambiense*. All treatments were kept in these conditions throughout a 65 days' period, at which moment a set of morphological, physiological and biochemical parameters was obtained. Well-watered plants, despite being infected with *N. kwambonambiense*, presented an overall photosynthetic increase, which enabled plant defense through the production of sugars, proline and salicylic acid. Oxidative damages (partially observed through malondialdehyde content), were avoided in part due to proline and soluble sugars. Water stress lead to a direct growth impairment confirmed through an indole-acetic-acid content decrease. A water-potential reduction occurred, which, together with abscisic acid, lead to stomatal closure and overall photosynthetic efficiency decline. Oxidative damages weren't properly managed and further affected *E. globulus*. Furthermore, *N. kwambonambiense* was found to promote a jasmonic acid content increase, typical of necrotrophic pathogens, which may suggest a lifestyle change from hemibiotrophic to necrotrophic as plant cells progressively degenerate. Ultimately, water-stressed *E. globulus* presented larger external lesion extensions and steam cankers and a superior internal fungi progression. Our results conclusively demonstrate that water stress created a better substrate for fungi development and decreased the plant's ability to respond. Such resulted in higher susceptibility and disease severity confirming predisposition.

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Part I

Forest and climate change

Forests supply energy, building materials, and food, but also deliver services such as carbon storing, biodiversity hosting, and climate regulation (Trumbore et al., 2015). Although adapted to some level of disturbance, forests face new threats, with human-related intensification of stressors, such as climate change, as one of the most hostile ones (Thuiller et al., 2005; Trumbore et al., 2015).

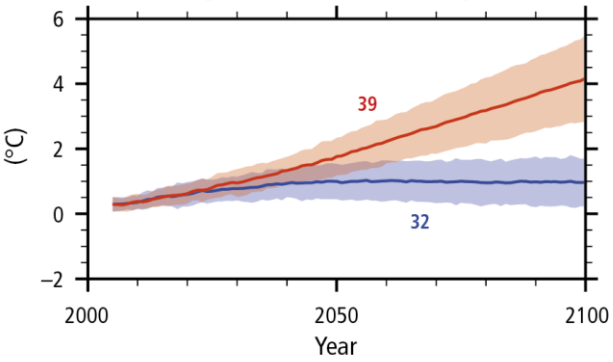

Figure 1 Global average surface temperature change from 2006 to 2100 determined by multi-model simulations under two scenarios RCP2.6 (blue) and RCP8.5 (red). Source: Pachauri et al., (2014)

From 1979 to 2010, global temperatures increased by about 0.5°C (Foster and Rahmstorf, 2011) and, according to the Intergovernmental Panel on Climate Change (IPCC) 2014 report, Earth's surface temperature is projected to continue to rise over the course of the 21st century (Pachauri et al., 2014) (fig.1), with more frequent temperature extremes and reductions in summer precipitation across Europe (Blenkinsop and Fowler, 2007; Dai et al., 2004). This combination will give rise to an increase of drought episodes, a major abiotic influence that greatly limits plant growth and development (Reddy et al., 2004). Additionally, climatic models predict that the Mediterranean area will be one of most affected regions (Giorgi, 2006), particularly, the northern Mediterranean, Lusitanian, and Mediterranean mountain regions (Thuiller et al., 2005).

Climate is an important distribution driver, predominantly regarding plant pathogenic fungi, being capable to give rise to new pathosystems (Desprez-Loustau et al., 2007; La Porta et al., 2008). It can exacerbate forest pathogens invasions, increasing the range of both native and exotic species (Jactel et al., 2012). This way, mild diseases, under normal conditions, can become severe under extreme weather conditions (Desprez-Loustau et al., 2006; Slippers and Wingfield, 2007). Such interaction gives rise to new and complex challenges to the eucalypt forest industry that are further discussed throughout this dissertation.

Eucalyptus spp. plantations

Despite constituting only 7% of the global forested area, planted forests significantly contribute to the overall forest's goods and services (FAO, 2015). *Eucalyptus* spp. belong to the *Myrtaceae* family, are native to Australasia, and comprise more than 700 species (Brooker and Hopper, 2002). *Eucalyptus* spp. are known for their rapid growth, short rotation time, and high adaptability to soils, climate, and nutrient conditions (Booth, 2013; Florence, 2004). For such reasons, are considered a major source of biomass for paper pulp production, being used for industrial charcoal and firewood production, and recognized as a potential source of biofuels and biomaterials (Luger, 2003; Turnbull, 1999). Therefore, it comes with no surprise that *Eucalyptus* spp. are the most widely planted hardwood, explored in plantations in over 90 countries, extending from southern Europe to South Africa, totaling more than 20 million hectares (Flynn, 2010; Iglesias-Trabado and Wilstermann, 2008).

Eucalyptus globulus

One of the most noteworthy *Eucalyptus* spp. species is *Eucalyptus globulus* (Labill.). In Europe, it covers 1.3 million ha, mainly in the Iberian Peninsula (more than 80%), France and Italy (Iglesias-Trabado and Wilstermann, 2008). In spite of the non-optimal conditions of the Mediterranean climate, it has become the most widely introduced species in the region (Navarrete-Campos et al., 2013).

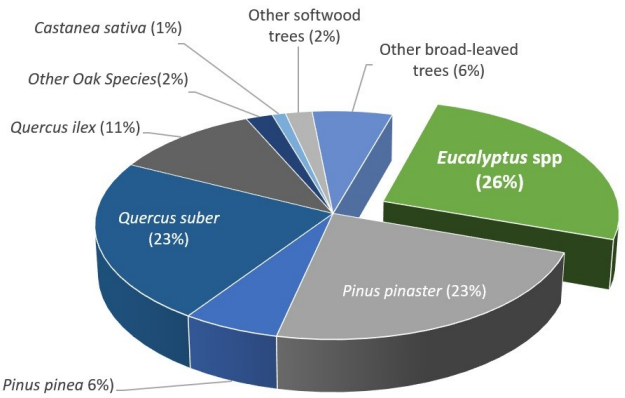

Figure 2 Forest area distribution by species/genera/group in mainland Portugal. Adapted from: ICNF (2013)

Eucalyptus globulus was introduced in Portugal around 1860, for timber and paper pulp production. Its expansion arose latter, in the mid-20th century, along with the paper and pulp industry growth (Borralho et al., 2007). The combination of breeding programs and improved techniques for cutting rooting led to the selection of clones taking into account their high growth rates, high pulp yield and environmental adaptability (Costa et al., 2004). Since its introduction, *E. globulus*' planted area has progressively increased (fig. 2), reaching 811,943 ha in 2010 (ICNF, 2013), establishing *E. globulus* as the dominant forest type in Portugal (ICNF, 2013). *E. globulus,* due to the pulp industry, represents a valuable contribution to the country's economy (CELPA, 2014). Although, as an exotic species, *E. globulus* has been free from most diseases, this situation has changed in the last 30 years (Bragança et al., 2015).

The family *Botryosphaeriaceae*

Members of the *Botryosphaeriaceae* (*Botryosphaeriales*, Ascomycota) fungal family were first described in the 1820's and include more than 2000 taxa (Slippers and Wingfield, 2007). Presenting a worldwide distribution, they occur on a wide range of monocotyledonous, dicotyledonous and gymnosperms hosts (Slippers and Wingfield, 2007) and can infect healthy plants, directly through lenticels, stomata or other openings, and be present in a latent state (Michailides, 1991; Slippers and Wingfield, 2007). Infections of woody hosts are believed to predominantly occur through horizontal transmission via spores (Slippers and Wingfield, 2007). Considered opportunistic host colonists, they hold the ability to live endophytically in their hosts, becoming pathogenic and triggering disease when the host is exposed to a predisposing stress condition, such as drought, which is the most frequently reported one (Mohali et al., 2007; Slippers and Wingfield, 2007; Van Niekerk et al., 2011). *Botryosphaeriaceae* are commonly linked to cankers and dieback, followed by broad kino production, and, in severe circumstances, tree death (fig. 3) (Pavlic et al., 2007).

Figure 3 Typical *Neofusicoccum* and *Fusicoccum* symptoms on *Eucalyptus* spp. (a) and (b): tree death; (c) and (d): internal damage. Source: Slippers and Wingfield, (2007)

Their high occurrence, dispersion aptitude, and ability to cause disease when their hosts are under stress, make these pathogens a significant threat to forests and plantations, particulary to non-native species (Crous et al., 2006; Slippers and Wingfield, 2007). In Portugal, according to Barradas et al. (2016), nine species from three different *Botryosphaeriaceae* genera were found in *E. globulus,* namely *Botryosphaeria dothidea*, *Diplodia corticola*, *Diplodia seriata*, *Neofusicoccum spp.*, *Neofusicoccum australe*, *Neofusicoccum algeriense*, *Neofusicoccum eucalyptorum*, *Neofusicoccum parvum*, and *Neofusicoccum kwambonambiense*.

Neofusicoccum kwambonambiense

Neofusicoccum species affect several woody species worldwide (Sakalidis et al., 2013). *Neofusicoccum kwambonambiense* (Pavlic, Slippers & M.J. Wingf.) is part of the *Neofusicoccum parvum – Neofusicoccum ribis* species complex, and is commonly found in its asexual stage (Pavlic et al., 2009).

Neofusicoccum kwambonambiense has been found on four continents, six countries and fourteen hosts (Sakalidis et al., 2013). Those including blueberry, in Florida (Wright and Harmon, 2010), *Syzygium cordatum* (Pavlic et al., 2007), *Cordia Africana* (Slippers et al., 2014), *Eucalyptus grandis* (Pillay et al., 2013) and *Acacia karro* (Slippers et al., 2009), in South Africa. *Eucalyptus dunnii* and *Corymbia torelliana*, in Australia (Sakalidis et al., 2011) and *Vitis vinifera*, in Uruguay (Abreo et al., 2013). From the nine *Botryosphaeriaceae* species detected in Portugal, in 2012, by Barradas et al. (2016), *N. kwambonambiense*, found causing stem canker (fig. 4), was considered to be the most aggressive. For such reason, *N. kwambonambiense* was the chosen pathogen to explore in this study.

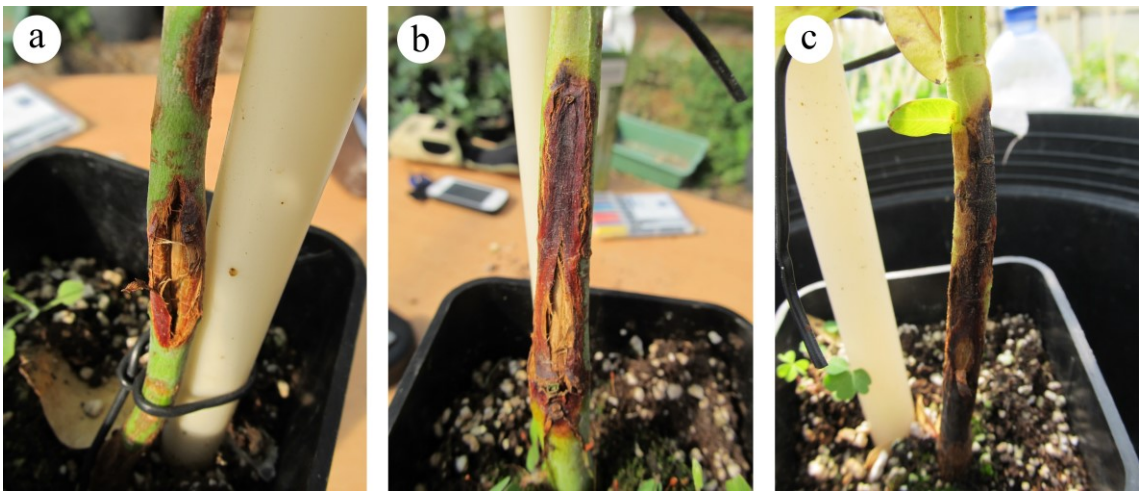

Figure 4 External lesion caused by *N. kwambonambiense* on *E. globulus x E. cypellocarpa* (a), *E. globulus* (b) and *E. nitens* (c) under a pot experiment. © Carla Barradas, 2014.

Quarantine systems have proven unsuccessful to control latent pathogens since they are able to be endophytically present in healthy plant material (Slippers and Wingfield, 2007), and are difficult to control once introduced into new areas (Swart and Wingfield, 1991). Chemical control is problematic on a large scale and removal or treatment of diseased tree parts, along with sanitation intended to reduce spore loads, appears to be the best approach to reduce disease (Brown-Rytlewski and McManus, 2000; Slippers and Wingfield, 2007; Stanosz et al., 2005). Nevertheless, symptoms are likely to continue to appear from other endophytic infections, and susceptible species can be replaced with resistant ones (Swart and Wingfield, 1991).

Drought-disease interaction effect on plant physiology

Plant predisposition and multiple stress concept

Interaction effects through host physiology can be allocated into two types of interaction: (1) combined effects of infection and drought on tree physiology: the “multiple stress concept”; and (2) an effect of drought on tree susceptibility to disease: the “predisposition concept” (Desprez-Loustau et al., 2006). Having in account that *N.* kwambonambiense is known to cause serious damage on plants previously subjected to drought, this study will focus on the predisposition effect.

The concept of predisposition, that is, the increased susceptibility to pathogen infection caused by environmental stress, was introduced and developed by Schoeneweiss (1978). Two main mechanisms may explain predisposition: (1) water stressed plant tissues are a better substrate for pathogen growth and (2): a decrease in plant active resistance may occur (Desprez-Loustau et al., 2006). This subject is of great scientific interest and has been reviewed (Bostock et al., 2014; Boyer, 1995; Desprez-Loustau et al., 2006). The great majority of published studies dealt with *Quercus*, *Populus* and *Pinus*, genus of considerable productive and environmental importance (Desprez-Loustau et al., 2006) and, conclusively, reported cases of water stress-caused predisposition. The hybrid *Populus nigra* × *Prunus maximowiczii* developed larger cankers as a result of *Septoria musiva* infection when grown under moderate drought (Franceschini et al., 2004); *Pinus resinosa*, under similar conditions, became more susceptible to *Sphaeropsis sapinea* infection (Blodgett and Stanosz, 1997); pistachio developed more severe *Botryosphaeria* blight disease (Ma et al., 2001); and, in field-grown grapevines, symptom development associated with *Botryosphaeriaceae,* was more severe in cases where plants have been, or still were, exposed to water-stress (Van Niekerk et al., 2011).

Plant response to drought and pathogen

Several investigations revealed that abiotic and biotic stressors can engage common signals and share responsive genes and products (Bostock et al., 2014). In recent years, research has largely focused on individual biotic or abiotic stress (Niinemets, 2010). Several studies regarding the effect of water stress on *Eucalyptus* spp. have been explored (Correia et al., 2016, 2014b; Li et al., 2000; Martorell et al., 2014; White et al., 1996a). Additionally, the distribution and effect of *Botryosphaeriaceae* on *Eucalyptus* spp. has also been addressed (Alves et al., 2013; Barradas et al., 2016; Pavlic et al., 2007; Pérez et al., 2010; Slippers and Wingfield, 2007). Nonetheless, the response to the collective drought and pathogen stress cannot be predicted based in the plant’s response to the individual stresses, being guaranteed to lead to a more complex scenario (Desprez-Loustau et al., 2006; Niinemets, 2010; Rejeb et

al., 2014). The link between these three elements is a clear example of a “disease triangle” (fig. 5). Introduced by Horsfall and Dimond (1960), the “disease triangle” is a plant pathology concept that studies the interactions between a biotic agent (*N. kwambonambiense*), a susceptible host (*E. globulus*), and a favorable environment for disease development (climate change driven-drought). This work intends to fill the knowledge gap that exists regarding this scenario from a predisposition point of view and, although in an interaction study all factors response should be explored, the focus of the study was narrowed to the *E. globulus* physiological response.

Biomass, growth and morphology

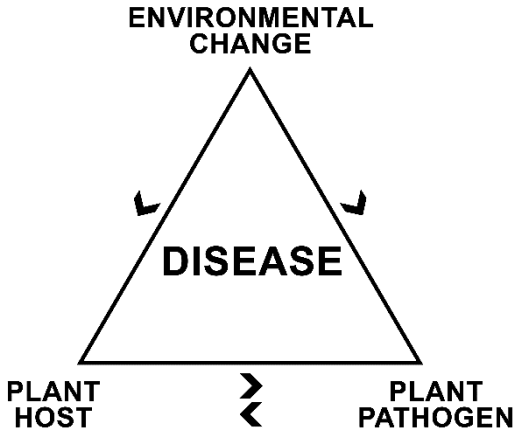

Figure 5 Modification of the environment-host-pathogen triangle highlighting the effect of environmental change on the interaction between plant host and plant pathogen. Adapted from: Grulke,(2011)

Plant growth and development impairment, as a result of turgor pressure reduction and decreased CO_2 diffusion, is one of the most drought sensitive and perceptible effects (Shao et al., 2008). Such causes water-stressed plants to often be smaller when compared to optimal watered ones (Souza and Cardoso, 2003), as found in *E. globulus* and other *Eucalyptus* spp. (Correia et al., 2014b; Costa et al., 2004; Li et al., 2000; Pita and Pardos, 2001; Shvaleva et al., 2006). Pathogens can also disturb both primary and secondary metabolism, affecting plant growth and development (Berger et al., 2007). Exposure to biotic and abiotic stresses thus leads to a disruption of the plant’s metabolism, with physiological costs and, ultimatly, to reductions in plant fitness and productivity (Rejeb et al., 2014).

Water relations and osmolyte accumulation

In order for the plant to absorb water, its water potential must be lower than that of the water supply. A reduction of the plant’s water potential tends to occur under water deficit conditions (Boyer, 1982), with several *E. globulus* studies reporting such a response (Correia et al., 2014b; Pita et al., 2003; Shvaleva et al., 2006; White et al., 1996b). Although water

content may have a direct effect on fungal growth within the host, most fungal pathogens display plasticity and can develop at water potentials well below the minimum for growth of most higher plants (Desprez-Loustau et al., 2006). For example, *Botryosphaeria dothidea* exhibited a remarkable tolerance to low water potentials (Ma et al., 2001). In another work with *B. dothidea*, the author found that hyphae in vessels of stressed *Betula alba* stems were rectilinear and comparatively larger than the ones of unstressed plants (McPartland, 1984). Woody tissue desiccation leads to mechanical strength loss and can result in bark cracks that ease opportunistic endophytic pathogen invasion (Bettucci et al., 1999; Moore, 1987).

Osmotic adjustment is a physiological adaptation intended to maintain cell turgor and facilitate soil water extraction (Chen and Jiang, 2010; Morgan, 1984). It depends upon the accumulation of compatible solutes, particularly soluble sugars (Chen and Jiang, 2010; Couée et al., 2006). There is a strong correlation between water potential, soluble sugars concentration and stress tolerance. Drought stress leads to a water potential decrease and a soluble sugars accumulation (Morkunas and Ratajczak, 2014; Sperdouli and Moustakas, 2012). *E. globulus* showed an increase in sucrose upon water stress (Merchant et al., 2006). Additionally, soluble sugars have been found to express a signalizing function, similar to hormones, upon pathogen interaction, regarding the induction of pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) (Horacio and Martinez-Noel, 2013; Rojas et al., 2014; Rolland et al., 2006). Sugars are closely related to the abscisic acid signaling cascade, with such association leading to signal amplification (Pinheiro and Chaves, 2011). Thus, an effective immune response depends of a synchronized interaction of hormonal and sugar pathways that, under drought, may be severely impaired (Bolouri and Van den Ende, 2012). Proline is an amino acid essential for plant primary metabolism (Szabados and Savoure, 2010). This osmolyte is produced in response to stress, particularly drought (Choudhary et al., 2005; Sperdouli and Moustakas, 2012), protecting plant cells by osmotic adjustment and membrane stabilization (Sperdouli and Moustakas, 2012). Shvaleva et al. (2006) found an increase in proline concentration in water-stressed *E. globulus.* Proline has also been attributed an antioxidant role, suggesting a ROS scavenging activity (Matysik et al., 2002; Smirnoff and Cumbes, 1989).

Photosynthetic performance and related pigments

Photosynthesis is amongst the primary processes to be affected by drought (Xu et al., 2010). Such may occur as a result of two mechanisms: (1) directly, through a CO_2 availability decrease, as a consequence of stomatal closure (Chaves et al., 2009) and metabolic impairment (Reddy et al., 2004); and (2), indirectly, and typical of multiple stress conditions,

as a result of secondary effects, specifically oxidative stress (Chaves et al., 2009; Chaves and Oliveira, 2004). In addition to the CO_2 diffusion reduction through the stomata, drought leads to a CO_2 mesophyll conductance and transpiration decline (Flexas et al., 2004; Lima et al., 2003). Guarnaschelli et al. (2006) showed a stomatal conductance decline on water-stressed *E. globulus* seedlings. Lima et al. (2003) had similar results in several *Eucalyptus* species. Photosynthesis efficiency decline causes a reduction in ATP production. That results in a decreased synthesis of enzymes and proteins that are involved in pathogen defense (e.g. enzymes with a lytic action on fungal hyphae and phytoalexins). Such ultimately leads to an increased pathogen susceptibility and disease severity (Desprez-Loustau et al., 2006; Kruger and Manion, 1994; Van Niekerk et al., 2011).

Chlorophylls content is positively associated with photosynthetic rate (Anjum et al., 2011). Drought is known to lead to a loss of chlorophyll content, as the plant is forced to divert the absorbed light to other processes, like thermal dissipation to protect the photosynthetic apparatus (Reddy et al., 2004). Nevertheless, such reaction is not identical in all plants. When *Picea abies* seedlings were exposed to mild and severe drought stress, chlorophyll content was not affected (Ditmarová et al., 2009). Similar works with *Eucalyptus* spp. showed a chlorophyll content increase upon water stress (Correia et al., 2014b; Michelozzi et al., 1995). Carotenoids are involved in stress response as photoprotectors and ROS scavengers (Cazzonelli, 2011; Ismail et al., 2011). These natural pigments are also engaged in osmotic adjustment, preserving photosynthesis (Anjum et al., 2011). Furthermore they assist as precursors for abscisic acid biosynthesis (Cunningham Jr and Gantt, 1998).

Downregulation of photosystem II (PSII) activity gives rise to a discrepancy between the generation and use of electrons (Reddy et al., 2004). This effect can be analyzed by monitoring *in vivo* chlorophyll fluorescence. A method that depends upon the chlorophyll *a* fluorescence measurement in a dark adapted plant and after saturating light pulses (Schreiber, 2004). Such is a non-invasive and valuable tool for plant stress detection and photosynthesis assessment (Krause and Weis, 1991). Furthermore, as reviewed by Berger et al. (2007), chlorophyll fluorescence has proven effective in detecting photosynthetic disturbances before visible leaf damage occurred. However, the electron transport chain and its related processes are both resistant to drought stress (Ditmarová et al., 2009) and PSII activity may not decrease in a drought-dependent way (Sperdouli and Moustakas, 2012). Susiluoto and Berninger (2007) found an increased fluorescence in *Eucalyptus microtheca* under drought conditions and Correia et al. (2014b) had similar results with *E. globulus*. Down-regulation of effective photosystem II quantum yield was also found in several plant-pathogen interactions (Bonfig et

al., 2006; Chou et al., 2000; Swarbrick et al., 2006). Heat dissipation in the PSII core and antenna generate reactive oxygen species, leading to drought-induced oxidative stress (Reddy et al., 2004).

Oxidative response

Upon water stress-driven photosynthesis reduction, the excess excitation energy in photosystem II leads to a deficiency of the photosynthetic process with a consequent reactive oxygen species (ROS) accumulation (Aranjuelo et al., 2011; Chaves et al., 2009; Wilhelm and Selmar, 2011). Reactive oxygen species can react with DNA, lipids, and proteins, resulting in mutations and lipid/protein oxidation that lead to cell death and progressive organism aging (Beckman and Ames, 1998). ROS presence can be partially extrapolated through malondialdehyde (MDA) accumulation, a product of membrane lipid peroxidation (Cakmak and Horst, 1991). Plant pathogen recognition prompts a biphasic ROS accumulation with a rapid, but weak, transient phase, followed by a massive and prolong second phase known as “oxidative burst”. Such is a front-line defense mechanism essential for further defense reactions (Heller and Tudzynski, 2011; Lamb and Dixon, 1997). Furthermore, ROS function as both localized and distance signals, promoting phytohormone accumulation, particularly, salicylic acid, and pathogenesis-related gene transcripts (Bostock et al., 2014; Naidoo et al., 2014).

Phytohormones

Phytohormones, besides controlling plant growth and development, also regulate the plant response to biotic and abiotic pressures (Bostock et al., 2014; Peleg and Blumwald, 2011). Several phytohormones are involved in the amplification of the initial defense signal, taking part in the exceptionally tailored response to the invader (Glazebrook, 2005; Naidoo and Za, 2013; Naidoo et al., 2011). Each of these signaling cascades encompasses a pathogenesis-related (PR) genes stimulation, intended to limit pathogen development and induce plant protection (Naidoo et al., 2011). Defense- or pathogenesis related (PR) proteins are an important part of the plant’s immune system, providing rapid host protection with low energy cost (Lay and Anderson, 2005).

Abscisic acid (ABA) is considered an universal hormone that regulates numerous developmental processes and adaptive stress responses in plants (Jiang and Hartung, 2008). This phytohormone is hoarded in root tissues and, upon water stress, is released into xylem vessels and transported to the shoot where, along with water potential reduction, induces stomatal closure (Jiang and Hartung, 2008; Rejeb et al., 2014). Such leads to a reduced water

loss via transpiration and, eventually, to a restriction of cellular growth (Peleg and Blumwald, 2011). Correia et al. (2014) and Jesus et al., (2015) found that, upon water stress, *E. globulus* plants present significantly higher levels of ABA. Abscisic acid has also been found to be a complex modulator of plant defense responses against phytopathogens (Denancé et al., 2013; Rejeb et al., 2014). It can act as a positive or negative regulator of plant defense, depending on the plant-pathogen interaction; it can have a negative interaction with salicylic and jasmonic acids under the combination of biotic and abiotic stresses (Denancé et al., 2013; Rejeb et al., 2014). Mauch-Mani and Mauch (2005) found that ABA can be implicated in increasing resistance of plants towards pathogens via its positive effect on callose deposition, an inducible defense that can prevent pathogen invasion (Rejeb et al., 2014).

Indole-3-acetic acid (IAA) is the predominant plant auxin involved in plant growth and development being found to promote stomatal opening (Peleg and Blumwald, 2011; Teale et al., 2006). Indole-3-acetic acid also plays a role in plant-pathogen interaction. Many plant pathogens can produce auxins or influence host auxin biosynthesis to interfere with the host's developmental processes (Wang et al., 2007). Nevertheless, plants developed mechanisms to repress auxin signaling during infection, as a defense strategy, particularly through salicylic acid production that, in turn, downregulates IAA production and signaling (Wang et al., 2007).

Salicylic acid (SA) is a phenolic compound and an important phytohormone found to be involved in several physiological processes such as seedling development, stomatal aperture, senescence, respiration and cell growth (Janda and Ruelland, 2015). It was also found to increase in content in response to drought (Hayat et al., 2010). For example, *Phillyrea angustifolia* and barley, showed an increase in SA concentration in response to drought (Bandurska, 2005; Munné-Bosch and Peñuelas, 2003). Pathogen infection leads to SA accumulation in uninfected leaves, suggesting an involvement in systemic acquired resistance (SAR) (An and Mou, 2011; Gaffney et al., 1993). Such is a mechanism that grants plant protection against a broad spectrum of pathogens at sites distant from the pathogen entry point (Janda and Ruelland, 2015). This hormone was identified as a main signaling molecule required for the plant's local defense responses against biotrophic pathogen attack (Denancé et al., 2013; Halim et al., 2006). Pathogen infection incites a SA levels increase in cells surrounding infection sites (Enyedi et al., 1992) to give rise to the hypersensitive response (HR). Such, is a locally triggered mechanism that leads to the collapse and death of infected cells (Alvarez, 2000). This cell death may control pathogens that infect living cells, but can fail to restrict necrotrophic pathogens (Govrin and Levine, 2000).

While biotrophic pathogen defense depends on programmed host cell death, necrotrophic pathogens benefit from that mechanism and are, thus, targeted by the induction of the jasmonic acid pathway (Glazebrook, 2005). Jasmonic acid (JA), and its metabolites, collectively known as jasmonates, were found to be involved in plant responses to abiotic as well as to biotic stresses (Ghasemi Pirbalouti et al., 2014). Jasmonic acid was found to have an effect on abiotic stress in strawberry (Wang, 1999), pear (Gao et al., 2004) and *E. globulus* (Correia et al., 2014b). Furthermore, the JA/ET pathway is also involved in the stimulation of the induced systemic resistance (ISR) (Naidoo et al., 2014). Such results in priming, the induction of cellular defense responses in tissues distal to the point of stimulation (Pozo et al., 2008).

Induced responses to pathogens: A crosstalk mechanism

The first line of defense against phytopathogens is preformed (constitutive). It is present in host cells prior to pathogen contact, being considered a nonspecific shield (Oghenekaro et al., 2013). Once preformed defenses are breached, induced responses are activated (Jones and Dangl, 2006). These require recognition of non-self-cells by general elicitors called PAMPs (Pathogen Associated Molecular Patterns) or MAMPs (Microbe Associated Molecular Patterns), subsequent signaling and production of defensive products (Naidoo et al., 2014; Trouvelot et al., 2014). These patterns (MAMPs and PAMPs) are detected by pattern recognition receptors (PRRs), that lead to the non-specific immune response known as “pattern-triggered immunity” (PTI) (Dardick and Ronald, 2006; Morkunas and Ratajczak, 2014; Naidoo et al., 2014). Such immunity mechanism depends on MAP kinase activation, ROS production, transcriptional reprogramming, hormone biosynthesis and deposition of callose in the cell wall (Jones and Dangl, 2006; Morkunas and Ratajczak, 2014). According to some studies, cross-species PRRs could be potentially used to improve *Eucalyptus* spp resistance, being, therefore, attractive targets for manipulation (Naidoo et al., 2014).

Some pathogens have developed strategies to avoid or even suppress the plant’s PTI (Denancé et al., 2013). To counteract this, a second line of defense, more specific, rapid, and effective, known as “effector-triggered immunity” (ETI) takes action (Dodds and Rathjen, 2010). It depends of the recognition of effectors by plant resistance proteins (PRP) (Bolouri and Van den Ende, 2012; Naidoo et al., 2014) and involves mechanisms such as oxidative burst and hypersensitive response, being associated with extensive changes in gene expression (Naidoo et al., 2014). With the recent knowledge development on both processes, it has become more and more clear that both PTI and ETI are probably regarded as two

mechanisms of the same process (Boller and Felix, 2009). Nonetheless, this subject is still in its beginnings and additional studies should be performed.

The master dissertation: main purposes

The current climate change scenario, with its high extent and speed, is projected to induce direct growth impairment as well as hazardous changes in plant-pathogen interactions. Such is expected to increasing disease incidence and severity, particularly to *Eucalyptus* spp. plantations. Considering the fundamental importance of *E. globulus* and how drought increases the threat that *N. kwambonambiense* poses to plantations, it is of major concern to assess the predisposition effect that drought may have in *N. kwambonambiense* infection in order to provide insights that can be of service to the eucalypt forest industry.

The aim of this study was, on a secondary level, to evaluate the *Eucalyptus globulus* response to the individual drought and pathogen stresses and, on a primary level, to assess the predisposition effect that drought stress may have on *Eucalyptus globulus* infection by *Neofusicoccum kwambonambiense.* To achieve these objectives, a holist set of morphological, physiological, and biochemical traits were assessed, including fungal progression analysis, water relations, osmolyte accumulation, lipid peroxidation and photosynthetic and hormonal responses.

The master thesis disclosed herein is presented as a research paper and it is prepared according to the authors' instructions from the journal Environmental and Experimental Botany.

Part II

Physiological and hormonal changes behind *Eucalyptus* predisposition to *Neofusicoccum kwambonambiense* under water stress

Glória Pinto[1*], Helder Berenguer[1*], Barbara Correia[1], Aurelio Gómez-Cadenas[2], Andreia Cerqueira[1], Artur Alves[1#]

[1] Department of Biology & CESAM – Centre for Environmental and Marine Studies, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[2] Universitat Jaume I, Departmento de Ciencias Agrarias y del Medio Natural, 12071 Castelló de la Plana, Spain
*Authors with equal contribution
[#] corresponding author: artur.alves@ua.pt; tel +351 234 247 182; fax + 351 234 372 587

Abstract

Eucalyptus spp. plantation forestry has exponentially grown and currently represents a valuable economic commodity through the paper pulp and timber industry. *Neofusicoccum kwambonambiense* is an endophytic opportunist plant pathogen known to severely affect *Eucalyptus globulus*. Global temperatures have been increasing and this trend is expected to maintain in the future with expected losses to the eucalypt industry, directly, and as the result of the interaction between pathogens and climate change-driven drought. Taking such in consideration, it is of major importance to assess, on a physiological and hormonal levels, the predisposition effect that drought may have in *N. kwambonambiense* infection, in order to provide insights that may be of service to the eucalypt forest industry. To do so, *E. globulus* plants were inoculated with *N. kwambonambiense* after a 23-day water stress period (18% of field capacity). Physiological and biochemical parameters and hormonal content were assessed 65-days post-inoculation. Water-stress lead to a water potential decrease and a further ABA-triggered stomatal closure. Although hormonal (salicylic and jasmonic acid), oxidative and high-sugar-resistance responses took place, the plant was unable to properly react to oxidative damages (observable through malondialdehyde content) that, along with the already imposed gas restrictions, lead to a decrease in the photosynthetic efficiency. Such resulted in a compromising cycle of increased lesion extension and further susceptibility to infection. Ultimately a higher *N. kwambonambiense* progression and disease expression arose, therefore substantiating the predisposition hypothesis. This work fills a scientific gap, not only verifying the water stress predisposition effect but also exploring the plant's physiological response, unveiling plant fitness and its role on water stress predisposition.

Keywords: Forest trees, Plant performance, Biotic and abiotic interactions, Plant-pathogen interactions

Introduction

Forest tree growth, productivity and survival can be negativly affected by environmental stresses (Osakabe et al., 2012). Their effects are expected to become more pronunced in both duration and severity in the near future, partly do climate change and the occurence of simultaneous stress factors (FAO, 2015; Stella et al., 2013), particulary across Europe and the Mediterranean region (Blenkinsop and Fowler, 2007). Furthermore, according to several studies, water availability has been shown to be the main factor limiting plant growth, particularly of eucalypt plantations (Shvaleva et al., 2006; Villar et al., 2011).

Eucalyptus spp., known for their rapid growth, reduced rotation time and high adaptability to soils and climate, have become a key genera amongst planted hardwoods (Booth, 2013; Florence, 2004; Luger, 2003). Native to Australasia, they are explored in more than 90 countries, with main plantations located in Brazil, India and China, totaling more than 20 million ha (Brooker and Hopper, 2002; Iglesias-Trabado and Wilstermann, 2008). They represent a key source of biomass for fibreboard, industrial charcoal, fueldwood and paper pulp, being likewise recognized as a potential biofuels and biomaterials source (Hinchee et al., 2011; Luger, 2003; Turnbull, 1999). Acoording to the most recent Portuguese National Forest Inventory (ICNF, 2013), *Eucalypus* spp., particulary *Eucalyptus globulus* (Labill), occupys more than 800 000 planted ha, corresponding to 26% of the total forest area. *E. globulus* was introduced in Portugal around 1860 for timber and paper pulp production (Borralho et al., 2007) and, due to their high growth rates, pulp yield and environmental adaptability (Costa et al., 2004), have become the dominant forest type with a considerable economic importance through the paper pulp industry (CELPA, 2014).

Neofusicoccum kwambonambiense (Pavlic, Slippers & M.J) is part of the *Botryosphaeriaceae* spp. (*Botryosphaeriales*, Ascomycetes), a vast distribution family that occurs on a wide range of dicotyledonous, monocotyledonous and gymnosperm hosts (Crous et al., 2006; Slippers and Wingfield, 2007). *Botryosphaeriaceae* spp. are mostly considered opportunistic endophytes that, upon stressful conditions, such as hail, frost, insect damage, and, particularly, drought, adopt a pathogenic behavior (Crous et al., 2006). When affecting *Eucalyptus* spp., lead to shoot and branches dieback, stem cankers followed by kino exudation, coppice failure, and, in severe cases, tree death (Slippers et al., 2009; Slippers and Wingfield, 2007). Their high occurrence, dispersion aptitude and ability to cause disease when their hosts are under stress, makes this pathogen a significant threat to native forests and plantations, specially *Eucalyptus spp.*, where they are explored as non-native species (Crous et al., 2006; Slippers et al., 2004; Slippers and Wingfield, 2007). Barradas et al. 2016 detected,

in Portugal, in 2012, the first recorded occurrence of *N. kwambonambiense* in *E. globulus*, which was considered the most aggressive species amongst the other *Botryosphaeriaceae* species found.

There is a growing acknowledgement amongst the scientific community and policy makers that sustainable forest management is affected by multiple factors associated with global climate change (Noss, 2001).Although under field conditions, often two or more stresses are simultaneously or successively associated (Chelli-Chaabouni, 2014) most of the research has largely focused on individual biotic or abiotic stresses (Niinemets, 2010). Several studies regarding the effect of water stress on *Eucalyptus* spp. have been explored (Correia et al., 2016, 2014b; Li et al., 2000; Martorell et al., 2014; White et al., 1996a). Additionally, the distribution and effect of *Botryosphaeriaceae* on *Eucalyptus* spp. has also been addressed (Alves et al., 2013; Barradas et al., 2016; Pavlic et al., 2007; Pérez et al., 2010; Slippers and Wingfield, 2007). Nonetheless, the response to the collective drought and pathogen stress, from a predisposition point of view, cannot be predicted based in the plant's response to the individual stresses, being assured to lead to a more complex picture, particularly, under a climate change scenario (Desprez-Loustau et al., 2006; Niinemets, 2010; Rejeb et al., 2014).

The role of water stress has been object of predisposition-related research (Bostock et al., 2014; Desprez-Loustau et al., 2006). The predisposition phenomenon is the consequence of abiotic stresses occurring prior to infection, increasing plant disease susceptibility (Bostock et al. 2014). Furthermore, when hosts are subjected to drought preceding inoculation, symptom development tends to be more severe (Jactel et al., 2012; Van Niekerk et al., 2011). Forest-related studies regarding predisposition dealt with different pathosystems, including: *Quercus* spp.- *Botryosphaeria stevensii* and *Phytophthora cinnamomi* (Luque et al., 2002), *Populus nigra* × *P. maximowiczii* - *Septoria musiva* (Franceschini et al., 2004), *Pinus resinosa* - *Sphaeropsis sapinea* (Blodgett and Stanosz, 1997), *Eucalyptus* spp. - *Botryosphaeria dothidea* (Smith et al., 1994) and grapevines - *Botryosphaeriaceae* spp. (Van Niekerk et al., 2011), and conclusively reported increased susceptibility and symptom severity under predisposing water stress conditions.

The integration of plant responses to abiotic and biotic stress requires the involvement of multiple strategies and signals that may be common or specific to each type of stress (Bostock et al., 2014). The degree of plant susceptibility to pathogen attack depends on the host condition upon infection but also on the host's metabolic status, crucial for pathogen growth, as it appears that pathogens are capable to manipulate different aspects of plant

metabolism to achieve optimal growth conditions (Chen et al., 2010; Desprez-Loustau et al., 2006; Dodds and Rathjen, 2010). Adaptation to both abiotic and later biotic stress have an impact on primary metabolism homeostasis (Bolton, 2009; Chaves et al., 2003). In fact, defense-related components synthesis (metabolites and proteins) is energy demanding (Bolton, 2009), and may be compromised since drought potentially leads to a disturbance in the energy balance as stomatal conductance takes place and photosynthesis is reduced (Correia et al., 2016, 2014b; Desprez-Loustau et al., 2006). Stomatal closure depends on the accumulation of solutes and the phytohormone abscisic acid (ABA) (Correia et al., 2014b; Jesus et al., 2015). This point represents a node of convergence in the crosstalk between water stress and changes in disease susceptibility as ABA, a primary dehydration-responsive messenger (Jiang and Hartung, 2008), has been found to increase susceptibility or resistance to different pathogens (Mauch-Mani and Mauch, 2005; Rejeb et al., 2014). Such dual effect makes ABA a divisive molecule, whose effects depends of the environmental conditions (Luna et al., 2011). Furthermore, ABA may operate, or not, through an antagonistic relationship with important disease resistance signals such as salicylic acid, and jasmonic acid (Bari and Jones, 2009; Peleg and Blumwald, 2011). Reactive oxygen species (ROS) signaling (Miller et al., 2008) together with plant hormones therefore represent major components of the plant's response regulatory networks, underlying abiotic stress adaptation and pathogen recognition (Bostock et al., 2014; Peleg and Blumwald, 2011).

Increased losses to the eucalypt industry are expected, as a result of the interaction between climate change-driven drought and pathogen increased incidence (Sturrock et al., 2011). Taking into consideration *E. globulus* economic benefits, climate change effects on forest pathogens, particularly on *N. kwambonambiense* dynamics, this study intends to explore how water limitation affects *E. globulus* susceptibility to *N. kwambonambiense*. Physiological and hormonal changes will be assessed to unveil plant fitness and its role on water stress predisposed plants. By predicting a situation that under a climate change scenario may worsen a problem already encountered by forests, we aim to contribute to a better forest management and sustainability.

Materials and methods

Plant, fungal material and growing conditions

Neofusicoccum kwambonambiense (strain CAA755) (Fig. 1) belonging to A. Alves culture collection, Universidade de Aveiro, Portugal, was isolated from *E. globulus* stem cankers, maintained on ½ strength potato-dextrose-agar (PDA) medium (Merck, Germany) at environmental temperature (about 20–25 °C). Strains were subcultured one-week prior to inoculation.

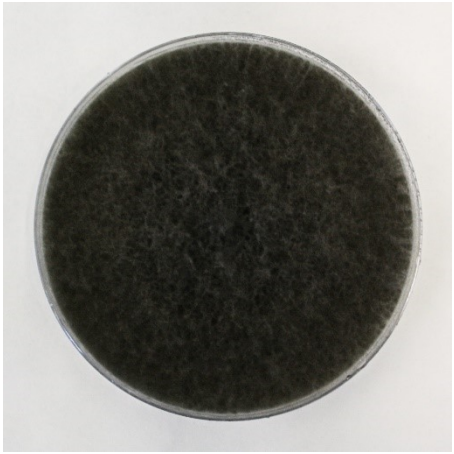

Figure 1 *N. kwambonambiense* grown on ½ strength PDA for one week

Four month old, *E. globulus* (clone GM-2-58) rooted cutting were obtained from Altri Florestal SA (Portugal), transplanted to 2 L plastic pots and filled with 3:2 (w/w) peat:perlite mixture. Potted cuttings were subjected to a 66-day acclimation period, watered on a regular basis to maintain 80% of field capacity (FC) and fertilized once a week with a (N:P:K 12:4:6) nutritive solution (FRUTIFOL L12®). To minimize the effects of environmental heterogeneity, pots were randomly arranged and sporadically repositioned to neighboring positions during the experiment. Pot weight was monitored gravimetrically. During the experimental period, daily mean air temperatures showed a progressive increase from 5-14°C (min.-max. temp.) in February, 2015 to 15-23°C (min.-max. temp.) at the end of the experiment, in July, 2015.

Experimental design

Plants were assigned to different water regime and fungi presence treatments (Fig. 2). Ten plants each made up the non-inoculated group, either well-watered (WW-NF), or water-stressed (WS-NF). Eighteen plants each made up the fungi-inoculated group, either well-watered (WW-F), or water-stressed (WS-F). WW plants were maintained in the same conditions of the acclimation period and WS plants were assigned to a water deficit regime of 18% FC. Such was achieved at the end of a 23-day period, by gradually reducing water supply. Once water-stressed was achieved, WW-F and WS-F plants were inoculated with *N. kwambonambiense*. To do so, a superficial wound, 1.5 cm long, was made on the stem of every plant with a sterile blade, 3-5 cm above ground level. Colonized PDA plugs, fungus-side-down, were placed on the wounds and immediately sealed with Parafilm® (American National Can, Greenwich, CT, USA). WW-NF and WS-NF plants stems received non-colonized PDA plugs. Plants were kept in these conditions throughout 65 days. To determine the time to end

the experiment, periodical external lesion extension measurements were made to identify the moment at which WS-F plants presented significantly higher values than WW-F plants.

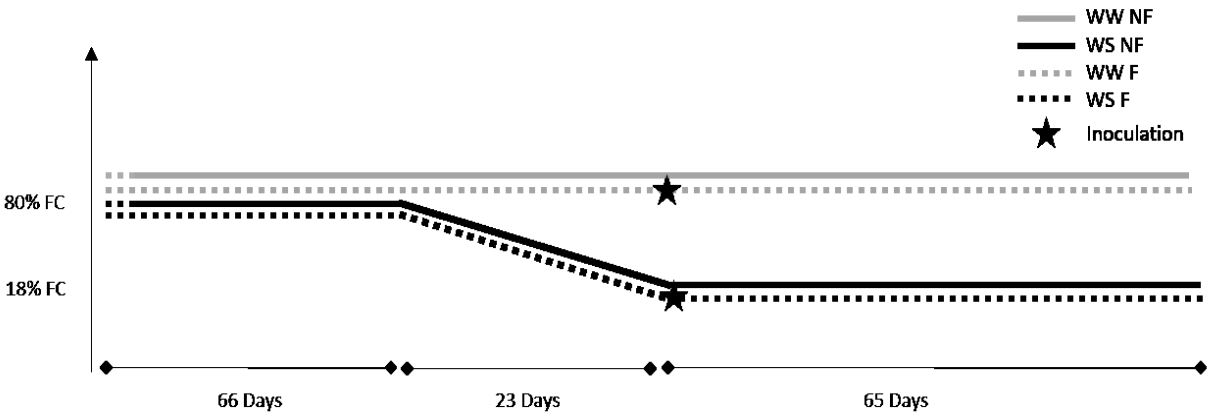

Figure 2 Experimental design implemented to evaluate the response of WW-NF, WS-NF, WW-F and WS-F, *E. globulus.* WW-NF: Well-watered, non-inoculated; WS-NF: Water-stressed, non-inoculated; WW-F: Well-watered, fungi-inoculated; WS-F: Water-stressed, fungi inoculated

Sample collection

Upon inoculation and till the end of the experiment, height and external lesion extension were periodically measured. At the end of the experiment (65 days-post inoculation (DPI)), the following *in situ* parameters were recorded in plants of each treatment group: external lesion extension, height, water potential, leaf gas-exchange and stomatal conductance parameters and chlorophyll *a* fluorescence. Also, fully expanded leaves were harvested and immediately frozen into liquid nitrogen for further biochemical analysis: total soluble sugars (TSS) and proline content, estimation of lipid peroxidation through malondialdehyde (MDA) quantification, chlorophylls *a,b* and carotenoid content and hormone quantification.

Height, external lesion extension and confirmation of *N. kwambonambiense* presence

Height (in cm), measured from the substrate surface to the apical meristem, was assessed in all plants. External lesion extension (in cm) was measured in all fungi-inoculated plants (both WW-F and WS-F). To confirm *N. kwambonambiense* presence, and confirm Koch's postulates, stem portions were extracted from the region where the lesion was made (L), in the intermediate point between the lesion and plant apex (L+) and near the plant apex (L++). Isolations were made by directly plating out stem portions on PDA medium (Merck, Germany) after surface sterilization. Plant tissue was sterilized by placing it in 5% sodium hypochlorite for 1 min, followed by 96 % ethanol for 1 min and rinsed in sterile water for 1 min. Plates were incubated at room temperature and checked regularly for fungal presence and

growth. Non-inoculated plant wounds (both WW-NF and WS-NF) successfully healed and no fungus was isolated from them.

Water status

Midday shoot water potential (Ψ_{md}) was measured in six randomly selected plants per treatment group with a Scholander-type pressure chamber (PMS Instrument Co., Corvallis, OR).

Leaf gas-exchange and stomatal conductance parameters

Intercellular CO_2 concentration content (C_i, ppm), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), and net CO_2 assimilation rate (A, µmol CO_2 m^{-2} s^{-1}) were performed using a portable infrared gas analyser (LC*pro*-SD, ADC BioScientific Ltd., UK) equipped with a broad leaf chamber. To determine the saturation light intensity A/PPFD (photosynthetic photon flux density; light response curves of CO_2 assimilation) curves were performed with the following PPFD: 2000, 1500, 1000, 750, 500, 250, 100, 50 and 0 µmol m^{-2} s^{-1}. After A/PPFD data analysis, punctual measurements at saturation light intensity were performed at 1500 µmol m^{-2} s^{-1}. The following conditions were maintained inside the chamber during all the measurements: air flux: 200 mol s^{-1}; block temperature: 25 ºC; and atmospheric CO_2 and H_2O concentration. Data were recorded when the measured parameters were stable (2–6 min).

Chlorophyll a fluorescence and photosynthetic pigments quantification

Steady-state modulated chlorophyll fluorescence was determined with a portable fluorometer (Mini-PAM; Walz, Effeltrich, Germany). Light adapted components of chlorophyll fluorescence were measured: steady-state fluorescence (F), maximal fluorescence (F'_m), variable fluorescence F'_v (equivalent to F'_m-F) and quantum yield of PSII photochemistry (φ PSII) equivalent to (F'_m -F)/F'_m. Leaves were then dark adapted for no less than 20 min to obtain F_0 (minimum fluorescence), F_m (maximum fluorescence), Fv (variable fluorescence, equivalent to F_m-F_0) and Fv/Fm (maximum quantum yield of PSII photochemistry).

Chlorophylls *a* and *b* and carotenoids content were quantified according to Sims and Gamon (2002). Pigments were extracted with acetone/Tris (50 mM) buffer at pH 7.8 (80/20) (v/v). After homogenization and centrifugation, supernatants were used to read absorbances at 663 nm, 537 nm, 647 nm and 470 nm (Thermo Fisher Scientific Spectrophotometer, Genesys 10-uv S, Waltham, MA). Chlorophyll *a* was calculated as (0.01373 A_{663} – 0.000897 A_{537} – 0.003046 A_{647}) and chlorophyll *b* as (0.02405 A_{647} – 0.004305 A_{537} – 0.005507 A_{663}).

Carotenoids content was calculated as [((A$_{470}$ – (17.1 x (Chl$_a$ + Chl$_b$) – 9.479 x B)) / 119.26], where B = (0.08173 A$_{537}$ – 0.00697 A$_{647}$ – 0.002228 A$_{663}$).

Lipid peroxidation

Lipid peroxidation on leaves was estimated by measuring the amount of malondialdehyde (MDA) which takes into consideration the possible effects of interfering compounds in the assay for thiobarbituric acid (TBA)-reactive substances using the method described by Hodges et al. (1999) with slight changes. Briefly, MDA was extracted from 50 mg frozen leaves using 1.25 mL of 80% (v/v) ethanol. After centrifugation, the supernatant was mixed with either 1 mL of 20 % TCA + 0.01% BHT and 20 % TCA + 0.5% TBA + 0.01% BHT and heated at 98 ºC for 30 m. Afterwards, samples were placed on ice for 5 m and absorbance was read at 600 nm, 532 nm and 440 nm against control. MDA equivalents (nmol ml^{-1}) were calculated as (A−B/157 000)×10^6, where A=[(Abs$_{532}$ + TBA) − (Abs$_{600}$ + TBA) − (Abs$_{532}$ – TBA − Abs$_{600}$–TBA)], and B=[(Abs$_{440}$ + TBA −Abs$_{600}$ + TBA) × 0.0571].

Total soluble sugars and proline quantification

Total soluble sugars (TSS) content was determined by the anthrone method, as described by Irigoyen et al (1992). Briefly, TSS were extracted from 100 mg frozen leaves using 10 mL of 80 % (v/v) ethanol at 80 ºC for 1 h. After centrifugation, supernatant was mixed with 1.5 mL of anthrone and incubated at 100 ºC throughout 10 min. Absorbance was read at 625 nm and TSS content was calculated against a D-glucose standard curve (0 to 1.25 mg/ml).

Proline content was determined as described by Khedr et al. (2003), with slight modifications. Briefly, about 100 mg of frozen plant material were homogenized in 1.5 ml of 3% sulphosalicylic acid, shaken vigorously and centrifuged at 10,000 *g* for 10 min at 4 °C. 1 ml of extract was incubated with 1 ml of ninhydrin acid and 1 ml of glacial acetic acid for 1 h at 100 °C. Afterwards, the reaction mixture was cooled in ice for 20 min. 1 ml of toluene was added and the mixture and left for 30 min until phases separation. Absorbance of the toluene phase was measured at 520 nm. Proline concentration was determined against a L-proline standard curve (0 to 50 μmol ml^{-1}).

Hormone quantification

Hormone extraction and analysis were carried out following the procedure described in Durgbanshi et al. (2005) with slight modifications. Frozen fresh tissue (0.2 g) was spiked with 100 ng of d6-ABA, 100 ng of Dihydrojasmonic acid, 5 ng of [2H$_2$] IAA, and 100 ng of d6-SA, prepared as in Gomez-Cadenas et al. (2002) and homogenized with 5 ml of distilled water.

After centrifugation at 4000 x g at 4 °C, supernatants were recovered and pH adjusted to 3 with 30 % acetic acid. The acidified water extract was partitioned twice against 3 ml of diethyl ether. The organic upper layer was recovered and vacuum evaporated in a centrifuge concentrator (Speed Vac, Jouan, Saint HerblainCedex, France). The dry residue was then resuspended in a 10 % MeOH solution by gentle sonication. The resulting solution was passed through 0.22 µm regenerated cellulose membrane syringe filters (Albet S.A., Barcelona, Spain) and directly injected into a UPLC system (Acquity SDS, Waters Corp., Milford, MA, USA).

Analytes were separated by reversed-phase (Nucleodur C18, 1.8 µm 50 × 2.0 mm, Macherey- Nagel, Barcelona, España) using a linear gradient of ultrapure H_2O (A) and MeOH (B) (both supplemented with 0.01 % acetic acid) at a flow rate of 300 µl min-1. The gradient used was: (0-2 min) 90:10 (A:B), (2-6 min) 10:90 (A:B) and (2-6-7 min) 90:10 (A:B). Hormones were quantified with a Quattro LC triple quadrupole mass spectrometer (Micromass, Manchester, UK) connected online to the output of the column through an orthogonal Z-spray electrospray ion source. Quantitation of plant hormones was achieved by external calibration with standards of known amount.

Statistical analysis

Student's t-test (t-test) was used to evaluate the effect of watering regime on the lesion extension of fungi inoculated plants (WW-F and WS-F). Two-way analysis of variance (ANOVA) was used to assess the effects of watering regime, fungi treatment and their interaction, on height, water potential, TSS, proline, gas exchange (ci, $_{gs}$, E, A), MDA, pigments (Chl *a*, *b* and carotenoids), chlorophyll *a* fluorescence and hormone content (ABA, IAA, JA, SA). Whenever an interaction between watering regime and fungi treatment was found, a simple main effect analysis (ANOVA one-way) was carried (using the error term of the two-way ANOVA as proposed by Quinn and Keough, (2002). When applicable, Holm-Sidak multiple comparison tests were employed to identify significant differences between treatments. T-test's, two-way ANOVAS and Holm-Sidak tests were performed using SigmaPlot (SigmaPlot for Windows v. 12.0, Systat Software Inc.) and main effects were calculated with MS Excel (Microsoft, Redmond, WA). Prior to analysis, data were ln- or 1/x-transformed to correct heteroscedasticity. (*) represent significant differences within water regimes, and (#) represent significant differences within fungi treatments All analyses used a significance level of 0.05.

Principal components analysis (PCA) was carried out to explore the morpho-physiological profile of *E. globulus* plants subjected to the different treatments by reducing the

multivariate data matrix to an interpretable bidimensional biplot that explains the highest proportion of variation in the data (ter Braak and Verdonschot, 1995). External lesion extension was excluded from the PCA because this parameter wasn't evaluated on non-fungi inoculated plants. PCA was conducted with Minitab 17 Statistical Software (2010). State College, PA: Minitab, Inc. Available from www.minitab.com.

Results

Height, external lesion extension and *N. kwambonambiense* presence confirmation

Water regime was the main influence on height differences (Fig. 3) (see F-ratios in Table 3) in a way that F plants did not differ from NF plants while WW plants consistently showed superior heights when compared with WS plants. At a morphological level, WS-F plants, besides presenting thinner stems, also presented larger internal lesion extensions (Fig. 4a). On an external level (Fig. 4b), at 58 DPI, WS-F external lesion extension was significantly larger than WW-F. To confirm whereas such difference was sustained, plants were kept for another 7 days, at which time, significant differences were maintained and the experiment came to an end. Moreover, *N. kwambonambiense* consistently reached superior heights in WS-F plants, 75% vs. 25%, for the L+ region and 25% vs. 0% for the L++ region (Table 1).

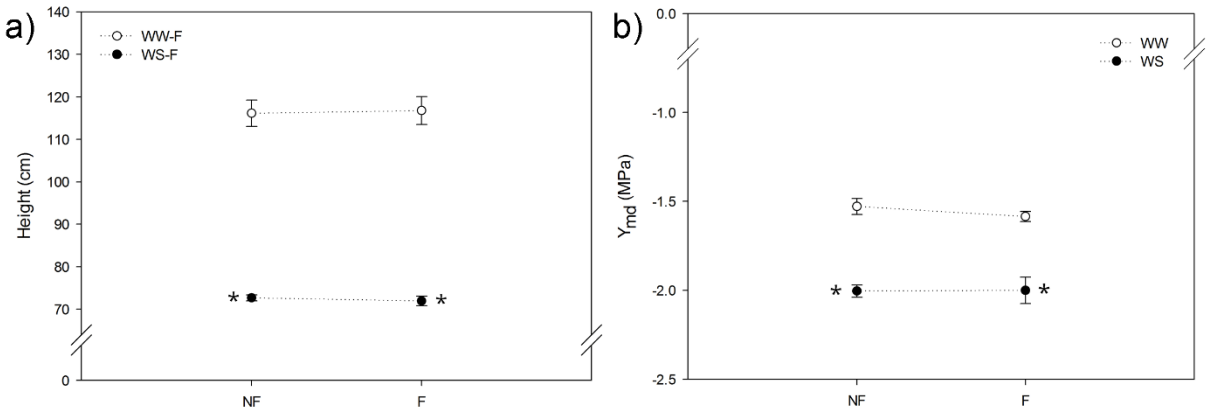

Figure 3 Height (a) and midday water potential (b) of of WW-NF, WS-NF, WW-F and WS-F *E. globulus*. Data are presented as mean ± SE. (*) indicate significant differences within water regimes and (#) indicate significant differences within fungi treatments. *($p \leq 0.05$).* WW-NF: Well-watered, non-inoculated; WS-NF: Water-stressed, non-inoculated; WW-F: Well-watered, fungi-inoculated; WS-F: Water-stressed, fungi inoculated

Table 1 Fungi presence in WW-F and WS-F *E. globulus* at different plant regions. In the region where the lesion was made and the fungi inoculated (L), between the lesion and plant apex (L+) and near the plant apex (L++). The presence of the fungi is shown in percentage of a total of 8 plants per treatment. WW-F: Well-watered, fungi-inoculated; WS-F: Water-stressed, fungi inoculated

Region/ Treatment	WW-F	WS-F
L++	0%	25%
L+	25%	75%
L	100%	100%

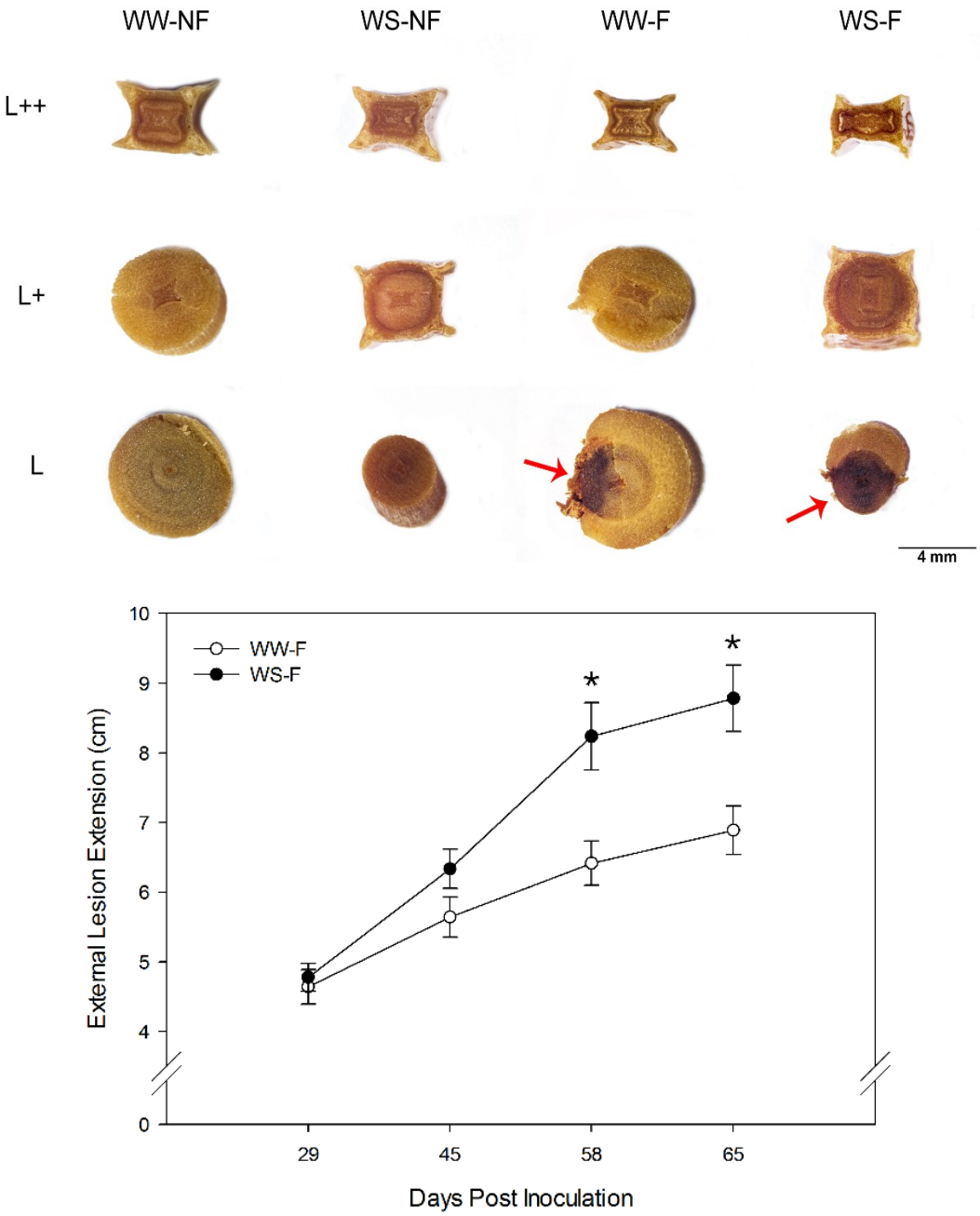

Figure 4 (a) Morphological aspect of WW-NF, WS-NF, WW-F and WS-F *E. globulus* stem cuts at different regionsIn the region where the lesion was made and the fungi inoculated (L), between the lesion and plant apex (L+) and near the plant apex (L++). Red arrows indicate lesion affected areas. (b) External lesion extension of WW-F and WS-F *E. globulus.* Data are presented as mean ± SE. (*) indicate significant differences within water regimes and (#) indicate significant differences within fungi treatments. *($p \leq 0.05$).* WW-NF: Well-watered, non-inoculated; WS-NF: Water-stressed, non-inoculated; WW-F: Well-watered, fungi-inoculated; WS-F: Water-stressed, fungi inoculated

Water status

Water regime was the main influence on water potential differences (Fig. 3b) (see F-ratios in Table 3) in a way that F plants did not differ from NF plants while WW plants consistently showed superior water potentials when compared with WS plants.

Leaf gas-exchange and stomatal conductance parameters

A significant interaction between water regime and fungi treatment was found in all gas exchange parameters (Fig. 5). Ci (Fig. 5a) presented a decrease upon water stress in NF plants and an increase in WS plants caused by *N. kwambonambiense* presence. Regardless of fungi presence, water stress caused a decrease in gs (Fig. 5b), E (Fig. 5c) and A (Fig. 5d) while an increase can be observed in WW plants as a result of interaction with *N. kwambonambiense*. Presence of *N. kwambonambiense* caused a decrease of E (Fig. 5c) and A (Fig. 5d) in already water-stressed plants. In all parameters, water regime was the main influence (see F-ratios in Table 3).

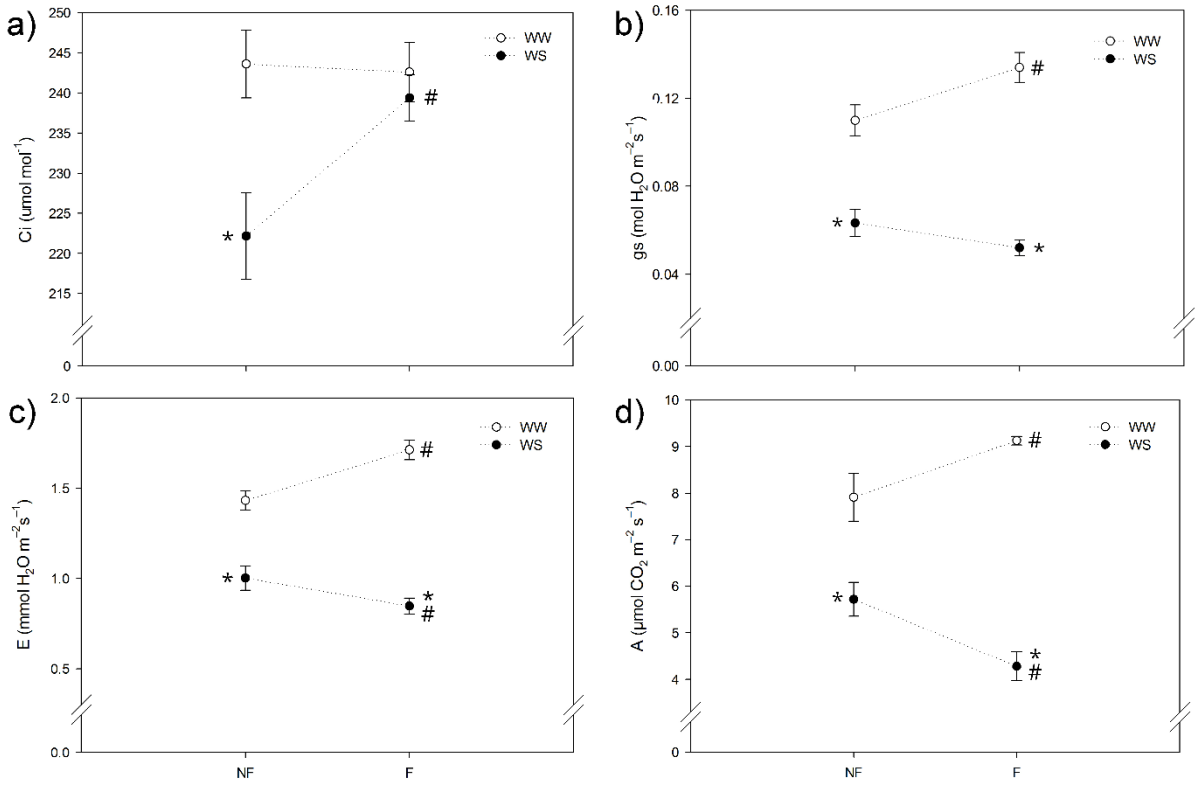

Figure 5 Intercellular CO_2 concentration (ci) (a), stomatal conductance (g_s) (b), transpiration rate (E) (c), and foliar net CO_2 assimilation rate (A) (d) of WW-NF, WS-NF, WW-F and WS-F *E. globulus*. Data are presented as mean ± SE. (*) indicate significant differences within water regimes and (#) indicate significant differences within fungi treatments. *(p ≤ 0.05).* WW-NF: Well-watered, non-inoculated; WS-NF: Water-stressed, non-inoculated; WW-F: Well-watered, fungi-inoculated; WS-F: Water-stressed, fungi inoculated

Chlorophyll a fluorescence and photosynthetic pigments quantification

No significant differences regarding Ch *a*, *b* and carotenoids concentration or F_v/F_m were found (Table 2). Only ΦPSII (Table 2) presented differences, with an increase in WW plants caused by *N. kwambonambiense* and a decrease in F plants caused by water stress. In this case, fungi presence was the main influence (see F-ratios in Table 3).

Table 2 Chlorophyll *a*,*b* and carotenoids content, maximum quantum yield (F_v/F_m) and effective quantum yield (ϕPSII) of WW-NF, WS-NF, WW-F and WS-F *E. globulus*. Data are presented as mean ± SE. (*) indicate significant differences within water regimes and (#) indicate significant differences within fungi treatments. *($p \leq 0.05$).* WW-NF: Well-watered, non-inoculated; WS-NF: Water-stressed, non-inoculated; WW-F: Well-watered, fungi-inoculated; WS-F: Water-stressed, fungi inoculated

Treatment	**Chl *a* content** (µmol g^{-1}DW)	**Chl *b* content** (µmol g^{-1}DW)	**Carotenoids content** (µmol g^{-1}DW)	**F_v/F_m**	**ϕPSII**
WW-NF	1.570 ± 0.124	0.576 ± 0.037	0.728 ± 0.045	0.823 ± 0.003	0.742 ± 0.002 #
WS-NF	1.574 ± 0.077	0.578 ± 0.079	0.691 ± 0.037	0.824 ± 0.003	0.748 ± 0.002
WW-F	1.760 ± 0.116	0.640 ± 0.051	0.815 ± 0.064	0.823 ± 0.001	0.760 ± 0.004#*
WS-F	1.738 ± 0.076	0.640 ± 0.027	0.742 ±0.027	0.826 ± 0.002	0.749 ± 0.004 *

Lipid peroxidation

A significant interaction between water regime and fungi treatment was found MDA content (Fig. 6a). Under WW conditions, *N. kwambonambiense* led to a decrease in MDA content. The effect was opposite in WS conditions, with an increase. A similar increase was identified in F plants under water stress conditions. In this case, water regime was the main influence (see F-ratios in Table 3).

Total soluble sugars and proline quantification

A significant interaction between water regime and fungi treatment was found in TSS (Fig. 6b) and proline content (Fig. 6c). Presence of *N. kwambonambiense* led to an increase of TSS in WW and WS conditions, although heightened in WS conditions. Accordingly, fungi treatment was the main influence (see F-ratios in Table 3). Presence of *N. kwambonambiense* led to an increase of proline content in WW conditions, as did water stress in NF plants. Water regime was, again, the main influence (see F-ratios in Table 3).

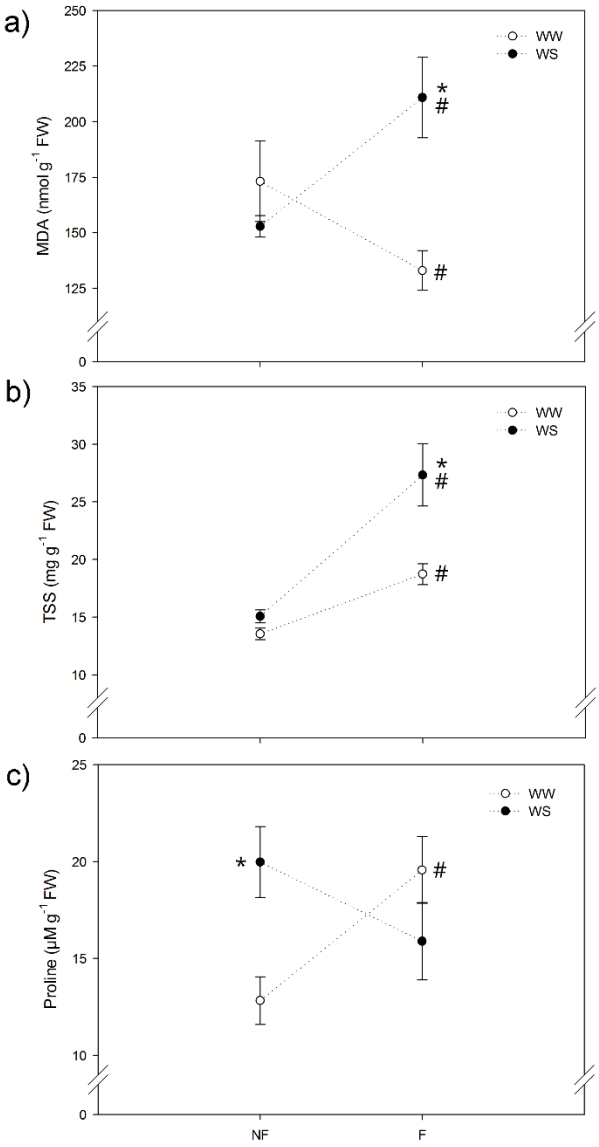

Figure 6 MDA (a), TSS (b) and proline (c) content in WW-NF, WS-NF, WW-F and WS-F *E. globulus*. Data are presented as mean ± SE. (*) indicate significant differences within water regimes and (#) indicate significant differences within fungi treatments. ($p \leq 0.05$). WW-NF: Well-watered, non-inoculated; WS-NF: Water-stressed, non-inoculated; WW-F: Well-watered, fungi-inoculated; WS-F: Water-stressed, fungi inoculated

Hormones

Regardless of *N. kwambonambiense* presence, water stress caused an increase in ABA content (Fig. 7a). In IAA content (Fig. 7d), a decrease is observed in NF plants. In both ABA and IAA content, water regime was the main influence (see F-ratios in Table 3). Water stress caused a decrease of JA (Fig. 7b) content in NF plants while in WS conditions, fungi inoculation led to an increase. Furthermore, a significant interaction between water regime and fungi treatment can be seen in JA content. In all treatments, both water stress and *N. kwambonambiense* caused an increase in SA

content (Fig. 7c). In both JA and SA content, fungi presence was the main influence (see F-ratios in Table 3).

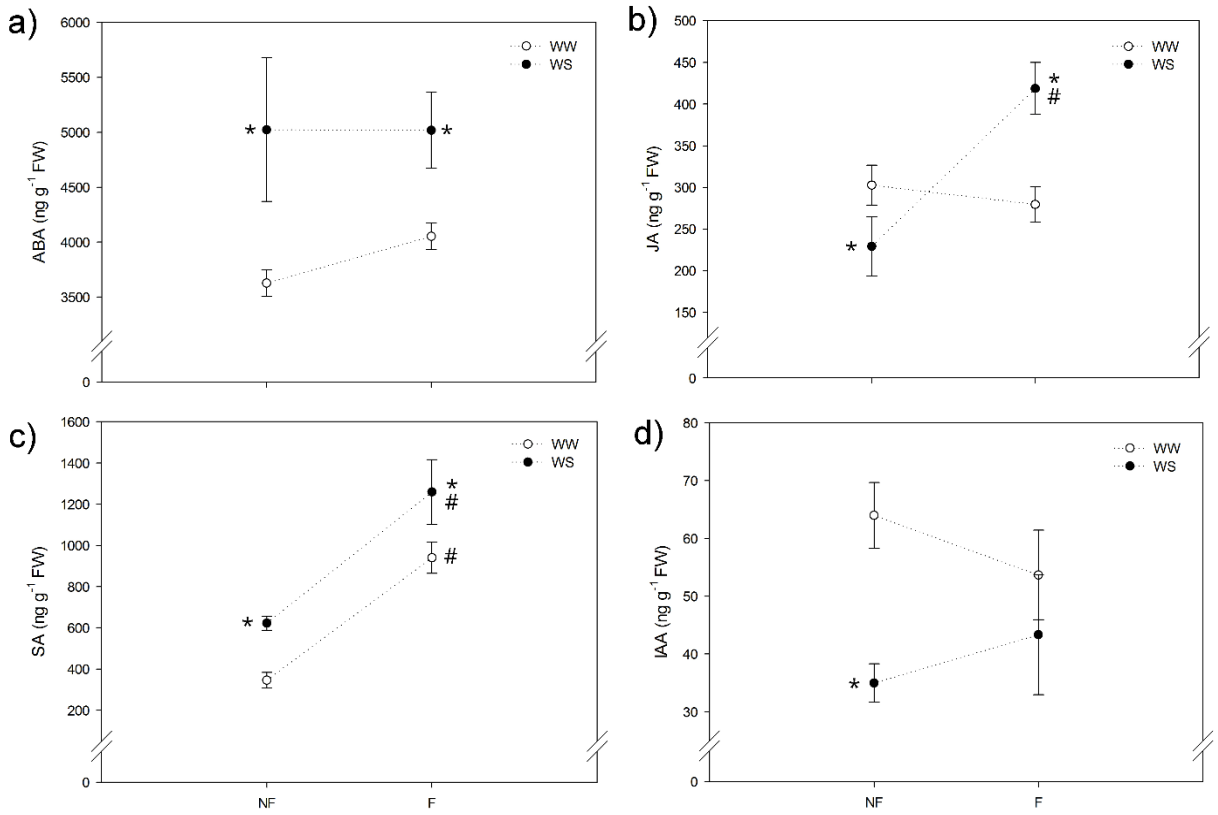

Figure 7 ABA (a), JA (b), SA (c) and IAA (d) content of WW-NF, WS-NF, WW-F and WS-F *E. globulus.* Data are presented as mean ± SE. (*) indicate significant differences within water regimes and (#) indicate significant differences within fungi treatments. *($p \leq 0.05$).* WW-NF: Well-watered, non-inoculated; WS-NF: Water-stressed, non-inoculated; WW-F: Well-watered, fungi-inoculated; WS-F: Water-stressed, fungi inoculated

Multivariate approach: a global overview of physiological status

Principal components analysis ordination (PCA) (fig. 8) provides a holistic overview of the experimental setup, revealing a separation between water regimes and fungi treatment. Well-watered plants (WW-NF and WW-F) are mostly grouped together (upper left section) suggesting homogeneity in the biochemistry and physiology response due to higher water potential, gas-exchange parameters (A, gs, E), height and IAA. Water-stressed plants are located in the right biplot section, with non-inoculated plants on the inferior half, due to high proline and ABA content, and fungi-inoculated plants on the upper section due to higher MDA, TSS, SA and JA content.

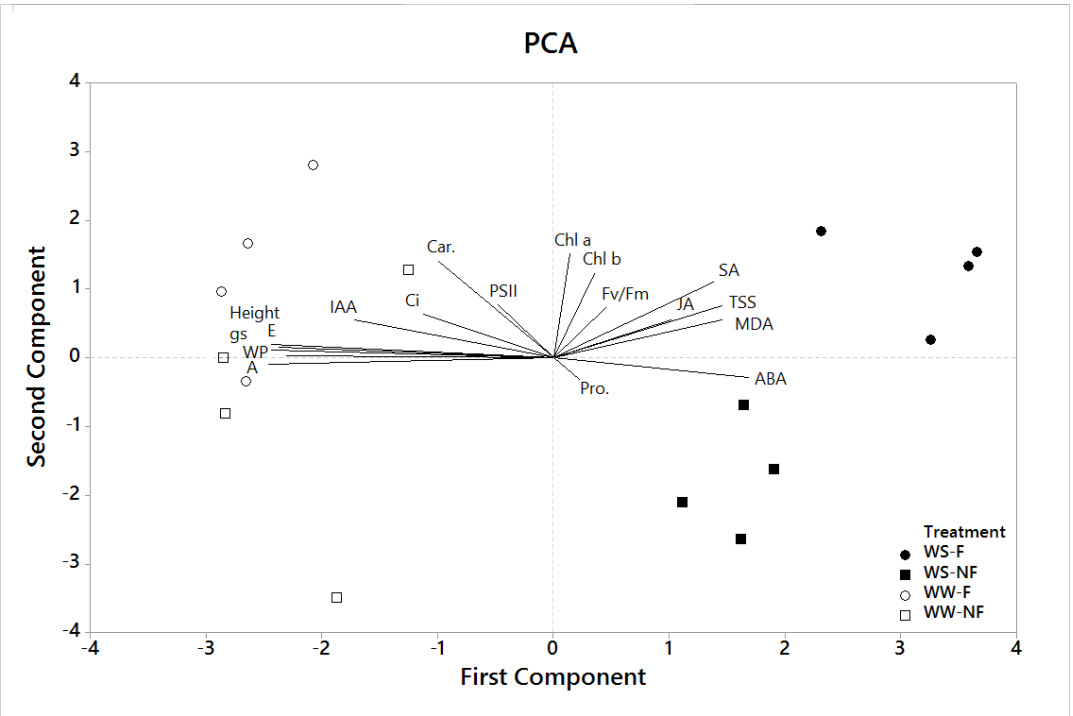

Figure 8 PCA biplot of the physiological data of *E. globulus* during the experimental setup. Different colors are used to differentiate well-watered (white) from water-stressed (black) plants while different symbols are used to differentiate non-inoculated (square) from fungi-inoculated (circle) plants. WW-NF: Well-watered, non-inoculated; WS-NF: Water-stressed, non-inoculated; WW-F: Well-watered, fungi-inoculated; WS-F: Water-stressed, fungi inoculated. Circles are used to highlight the position of specific scores (WW plants; WS-NF and WS-F plants)

Table 3. Two-way ANOVA summary table for morphological and physiological traits of WW-NF, WS-NF, WW-F and WS-F E. globulus. Degrees of freedom (d.f.) and F value are shown for each source of variation; variance (MS) of the residual is also shown. * P ≤ 0.05, **P ≤0.01, ***P ≤ 0.001, NS, non-significant

Parameter		Source of Variation (Sv)	df (Sv, Residual)	F	Significance	
Height (ln)		Water Regime	1,43	236.608	<0.001	***
		Fungi	1,43	0.0373	0.848	**NS**
		Treatment				
		Interaction	1,43	0.0359	0.851	**NS**
				MS = 0.00884		
Water Potential		Water Regime	1,21	153.441	<0.001	***
		Fungi	1,21	0.532	0.474	**NS**
		Treatment				
		Interaction	1,21	0.715	0.407	**NS**
				MS = 0.00802		
TSS Content		Water Regime	1,13	13.188	0.003	*
		Fungi	1,13	39.056	<0.001	***
		Treatment				
		Interaction	1,13	6.449	0.025	*
				MS = 8.204		
Proline Content		Water Regime	1,9	1.089	0.324	**NS**
		Fungi	1,9	0.640	0.444	**NS**
		Treatment				
		Interaction	1,9	10.620	0.010	**
				MS = 8.837		
Gas-Exchange	**Ci**	Water Regime	1,22	8.870	0.007	**
		Fungi	1,22	3.852	0.062	**NS**
		Treatment				
		Interaction	1,22	4.860	0.038	*
				MS = 102.620		
	E	Water Regime	1,22	129.900	<0.001	***
		Fungi	1,22	1.216	0.282	**NS**
		Treatment				
		Interaction	1,22	14.627	<0.001	***
				MS = 0.0194		
	gs	Water Regime	1,22	129.664	<0.001	***
		Fungi	1,22	1.257	0.274	**NS**
		Treatment				
		Interaction	1,22	9.778	0.005	**
				MS = 0.000192		
	A	Water Regime	1,22	90.177	<0.001	***
		Fungi	1,22	0.0908	0.766	**NS**
		Treatment				
		Interaction	1,22	12.795	0.002	**

				MS = 0.824		
Fluorescence	PSII	Water Regime	1,16	0.583	0.456	**NS**
		Fungi	1,16	9.132	0.008	*
		Treatment				
		Interaction	1,16	7.561	0.014	*
				MS = 0.0000455		
	Fv/Fm	Water Regime	1,22	0.611	0.443	**NS**
		Fungi	1,22	0.0862	0.772	**NS**
		Treatment				
		Interaction	1,22	0.234	0.634	**NS**
				MS = 0.0000338		
Pigment Content	Chl *a*	Water Regime	1,12	4.918	0.047	*
		Fungi	1,12	5.048	0.044	*
		Treatment				
		Interaction	1,12	0.00165	0.968	**NS**
				MS = 0.0233		
	Chl *b*	Water Regime	1,15	0.402	0.536	**NS**
		Fungi	1,15	3.787	0.071	**NS**
		Treatment				
		Interaction	1,15	0.0420	0.840	**NS**
				MS = 0.00645		
	Carotenoids	Water Regime	1,16	1.457	0.245	**NS**
		Fungi	1,16	2.342	0.145	**NS**
		Treatment				
		Interaction	1,16	0.158	0.696	**NS**
				MS = 0.0103		
MDA Content		Water Regime	1,12	4.359	0.059	**NS**
		Fungi	1,12	0.419	0.530	**NS**
		Treatment				
		Interaction	1,12	12.644	0.004	**
				MS = 763.14		
Hormones Content	ABA (1/x)	Water Regime	1,11	14.971	0.003	*
		Fungi	1,11	1.478	0.600	**NS**
		Treatment				
		Interaction	1,11	0.560	0.592	**NS**
				MS = 0.00000000163		
	SA (ln)	Water Regime	1,12	22.314	<0.001	***
		Fungi	1,12	81.361	<0.001	***
		Treatment				
		Interaction	1,12	2.736	0.124	**NS**
				MS = 0.0344		
	IAA	Water Regime	1,9	8.380	0.018	*
		Fungi	1,9	0.0217	0.886	**NS**
		Treatment				
		Interaction	1,9	1.887	0.203	**NS**
				MS = 147.704		

	Water Regime	1,12	1.332	0.271	**NS**
	Fungi	1,12	8.514	0.013	*
JA	Treatment				
	Interaction	1,12	13.942	0.003	**
			MS = 3246.438		

Discussion

Plants are often simultaneous exposed to multiple stress factors, both biotic and abiotic with different time framings, and, although available literature regarding combinatorial stress is limited, it is of suggestive of non-additive interactions with signaling mechanisms appearing to be partially shared (Kissoudis et al., 2014; Walley et al., 2007). Exposure to biotic and abiotic stress leads to a disruption of the plant's metabolism, with physiological costs and, ultimatly, to reductions in plant fitness and biomass productivity (Rejeb et al., 2014). The objective of this work was to assess the interaction between a biotic agent (*N. kwambonambiense*), a susceptible host (*E. globulus*) and a favorable environment for disease expression (climate change-driven drought) from a predisposition point of view. Our results conclusive reported that drought lead to predisposition to *N. kwambonambiense* infection in *E. globulus* with increased disease expression and severity.

Plant biomass reduction and growth impairment, perceptible through height, is one of the most noticeable and sensitive variables to be affected by water stress (Chaves and Oliveira, 2004; Shao et al., 2009). Plant growth rates were significantly decreased by water shortage, which is in agreement with other *E. globulus* reports (Correia et al., 2014b; Granda et al., 2011; McKiernan et al., 2014) while WW plants, regardless of *N. kwambonambiense* presence, kept consistently superior heights and corresponding IAA content, which is confirmed by the PCA analysis. Accordingly, water shortage lead to a decrease in midday water potential, as previously described in *E. globulus* (Correia et al., 2014b; Costa et al., 2004; Pita et al., 2003). Although plant water content may have a direct effect on fungal growth, most fungal pathogens are capable to adapt to low water potentials, well below the minimum for growth of most higher plants (Desprez-Loustau et al., 2006; Flowers et al., 2001). While no significant effects on water status due to *N. kwambonambiense* have been found, water-stress may lead to woody tissue desiccation that can result in bark cracks that facilitate pathogen invasion, predisposing the plant to infection while increasing symptom development (Bostock et al., 2014). Consistent with these results, is the extensive window of wound susceptibility found in stressed trees and not in well-watered ones (Mcintyre et al., 1996). Taking this into account, the larger external lesion extensions and higher fungi progression detected on water-

stressed plants can be considered the end result of an increased susceptibility to *N. kwambonambiense* caused by water shortage, also found in the interaction between *Botryosphaeria ribis* and *Eucalyptus* spp. (Old et al., 1990) and in other plant-pathogen interactions (Blodgett et al., 1997; Luque et al., 2002; Van Niekerk et al., 2011).

Water potential decrease leads to a ABA content upsurge, as seen in Correia et al., 2014b and Jesus et al., 2015, an important phytohormone which is transported to the shoot where it induces stomatal closure with costs to the photosynthetic apparatus (Jiang and Hartung, 2008). Stomatal closure leads to a decrease of the CO_2 availability (Chaves et al., 2003), that, together with oxidative stress (Jaleel et al., 2009) impair photosynthesis (Chaves et al., 2009; Lawlor, 2009). Simultaneously, a decrease of the CO_2 mesophyll conductance and transpiration takes place (Flexas et al., 2004). Our results demonstrate this interplay and report an ABA content increase, which is observed in similar *E. globulus* studies (Correia et al., 2014a; Granda et al., 2011) regardless of fungi treatment. Stomatal closure leads to an overall ci, gs, E and A reduction, being in agreement with Guarnaschelli, Prystupa, and Lemcoff 2006 and other reports (Bogeat-Triboulot et al., 2007; Correia et al., 2014b; Warren et al., 2011). Pathogen infection, under WS conditions, leads to a decrease in photosynthetic assimilate production (Berger et al., 2007; Bilgin et al., 2010), which is in agreement with other plant-pathogen interactions (Fontaine et al., 2016; Mayek-Pérez et al., 2002; Scharte et al., 2005; Swarbrick et al., 2006). The subsequent decrease in energy production results in a decay in enzymes and proteins synthesis that play an essential role in pathogen defense, increasing plant predisposition and exacerbating symptom severity (Van Niekerk et al., 2011). On the other hand, gas-exchange parameters increased on WW plants, along with effective quantum yield, with that being suggestive of an overall energy production increase to assure the plant protection. Photosynthetic rate is positively related with chlorophyll content (Anjum et al., 2011) and, although drought is known to lead to a chlorophyll content (Reddy et al., 2004), no content changes were found nor PSII photochemistry was negatively affected in response to water stress or *N. kwambonambiense* infection. Such may be the result of a series of compensatory reactions that mitigate the effects of water stress (Susiluoto and Berninger, 2007) protecting these pigments from degradation, while maintaining or even increasing its concentration as seen in several studies (Correia et al., 2014b; Michelozzi et al., 1995). Such depends upon the role of carotenoids actions as photo and osmoprotectores (Cazzonelli, 2011) and by soluble sugars overall osmotic adjustment mechanisms (Santakumari and Berkowitz, 1991). Furthermore, it reflects the plants ability to adapt to the established conditions, highlighting the need of long-term studies that take into account the acclimation effect (Zhou et al., 2016).

Soluble sugars, besides being involved in osmotic adjustment, have a crucial function as signaling molecules against pathogens through a “high-sugar resistance” mechanism (Morkunas and Ratajczak, 2014) and, upon pathogen infection, an increase of invertase activity takes place to meet such carbohydrate requirement (Roitsch and González, 2004). Soluble sugars are also involved in, or related to, ROS-producing metabolic pathways, protecting plants against photo-oxidation (Couée et al., 2006) whose content early increases upon pathogen infection to give rise to the oxidative burst (Heller and Tudzynski, 2011). Taking both mechanisms into account, the TSS increase upon *N. kwambonambiense* inoculation plants may be considered a direct response to pathogen infection, which is in agreement with other reports (Scharte et al., 2005; Swarbrick et al., 2006) being boosted as a result of water-stress (Couée et al., 2006; Rosa et al., 2009), and an indirect response to the supposed increase of ROS (Fontaine et al., 2016), observed through the high MDA content in the same water-stressed, *N. kwambonambiense*-inoculated plants. This is confirmed by the PCA that conclusively shows higher MDA content in water-stressed, *N. kwambonambiense*-inoculated plants. Nevertheless, water-stressed plants may be unable to properly respond to the harmful oxidative damages triggered by ROS.

Reactive oxygen species are also known to promote SA accumulation, therefore the SA content increase in WS-F was expected (Bostock et al., 2014; Naidoo et al., 2014) and corroborates its role regarding water stress (Hayat et al., 2010), as seen in *Phillyrea angustifolia* and barley (Bandurska, 2005; Munné-Bosch and Peñuelas, 2003) and pathogen local and systemic defense response (Denancé et al., 2013; Halim et al., 2006) as seen in *E. grandis* (Naidoo and Za, 2013). The increase in SA upon *N. kwambonambiense* inoculation in WW conditions may also be preventing an increase of JA in the same conditions, which supports the antagonistic crosstalk (Glazebrook, 2005) similarly detected in *E. grandis* (Naidoo and Za, 2013). Proline accumulation is also involved the response to water stress, thought osmotic adjustment, pathogen infection and overall free radicals scavenging (Chen and Jiang, 2010; Sperdouli and Moustakas, 2012; Szabados and Savoure, 2010). Therefore, the increase in proline upon water stress was expected and is in agreement with other reports (Cha-um and Kirdmanee, 2010; Li and Liu, 2004; Shvaleva et al., 2006), as is the increase in WW plants under *N. kwambonambiense* inoculation, which may reflect an active anti-oxidant response, as seen in other plant-pathogen interactions (Fabro et al., 2004; Haudecoeur et al., 2009). ABA may also have an antagonistic effect over JA (Denancé et al., 2013; Rejeb et al., 2014), which explains the decreased JA content in non-inoculated plants upon WS and is in agreement with other reports with *Eucalyptus globulus* (Correia et al., 2014a) and other plant-pathogen interactions (Anderson et al., 2004; Sánchez-Vallet et al., 2012). Furthermore, JA was found

to play a role in necrotrophic pathogen defense (Glazebrook, 2005; Halim et al., 2006), stimulating induced systemic resistance (Naidoo et al., 2014; Pozo et al., 2008), which explains its increase under WS conditions upon *N. kwambonambiense* inoculation, and is in agreement with other reports (Halim et al., 2004). It also suggests an hemibiotrophic lifestyle, with *N. kwambonambiense* switching from a biotrophic to a necrotrophic interaction, as seen in *Diplodia corticola* (Oliveira Fernandes, 2015) and further corroborates the higher tissue damages in WS plants as living tissue progressively degenerates.

Conclusions

Our results demonstrate that water stress-driven drought caused a decrease in the *E. globulus* ability to respond to *N. kwambonambiense* infection through a predisposition mechanism. Well-watered plants, despite being infected with *N. kwambonambiense,* presented an overall photosynthetic increase (A, PSII), which is suggestive of an active defensive response, through a presumable amplified production of enzymes, proteins and sugars (TSS) required to pathogen defense. *E. globulus* deployed an oxidative (MDA) and high-sugar response (TSS) to prevent pathogen expansion, which is observable through the inferior external and internal fungi expansion parameters. Despite that, oxidative damages were prevented through an active anti-oxidant machinery (TSS, proline) to protect the plant against damages. A concomitant increase of pathogen defense hormones content occurred, particularly salicylic acid, to lead to an efficient defense response, inferior fungi progression and consequent high-growth maintenance.

Water stress lead to a water-potential decrease, which triggered a ABA and TSS induced stomatal closure and overall photosynthetic efficiency decline. Although oxidative (observable through MDA) and high-sugars responses (TSS) took place, the plant was unable to control the resulting oxidative damages. Water potential decrease also induced tissue desiccation that, through a longer window of wound susceptibility lead to a concomitant larger external lesion extension and higher fungi progression. Moreover, and for the first time, under water-stress conditions, *N. kwambonambiense* was found to promote a JA content increase, typical of necrotrophic pathogens, which may suggest an hemibiotrophic lifestyle but also a change in the plant cells status.

This work fills a scientific gap regarding the effect that water stress, a phenomenon that is expected to increase as a result of climate change, on the predisposition of *E. globulus*, a tree established worldwide, with a pronounced economic value, to *N. kwambonambiense*, an opportunist endophytic highly aggressive phytopathogen. Furthermore, this works highlights

the need of long-term water stress studies that take into account the adaptation effects that take place in long-lived plants. Water stress recovery studies should take place to explore if the pathogen returns to a non-aggressive endophytic state, once water status is restored. This work provides insights about the *E. globulus* physiological and hormonal changes, unveiling plant fitness and its role on water stress predisposition that can be of use to the forestry industry.

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References

Abreo, E., Martinez, S., Bettucci, L., Lupo, S., 2013. Characterization of Botryosphaeriaceae species associated with grapevines in Uruguay. Australas. Plant Pathol. 42, 241–249. doi:10.1007/s13313-013-0200-8

Alvarez, M.E., 2000. Salicylic acid in the machinery of hypersensitive cell death and disease resistance. Plant Mol. Biol. 44, 429–442. doi:10.1023/A:1026561029533

Alves, A., Barradas, C., Phillips, A.J.L., Correia, A., 2013. Diversity of Botryosphaeriaceae species associated with conifers in Portugal. Eur. J. plant Pathol. 135, 791–804.

An, C., Mou, Z., 2011. Salicylic acid and its function in plant immunity. J. Integr. Plant Biol. 53, 412–428. doi:10.1111/j.1744-7909.2011.01043.x

Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R., Kazan, K., 2004. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis*. Plant Cell 16, 3460–3479.

Anjum, S.A., Xie, X., Wang, L.C., Saleem, M.F., Man, C., Lei, W., 2011. Morphological, physiological and biochemical responses of plants to drought stress. African J. Agric. Res. 6, 2026–2032. doi:10.5897/AJAR10.027

Aranjuelo, I., Molero, G., Erice, G., Avice, J.C., Nogués, S., 2011. Plant physiology and proteomics reveals the leaf response to drought in alfalfa (Medicago sativa L.). J. Exp. Bot. 62, 111–123. doi:10.1093/jxb/erq249

Bandurska, H., 2005. The effect of salicylic acid on barley response to water deficit. Acta Physiol. Plant. 27, 379–386. doi:10.1007/s11738-005-0015-5

Bari, R., Jones, J.D.G., 2009. Role of plant hormones in plant defence responses. Plant Mol. Biol. 69, 473–488.

Barradas, C., Phillips, A.J.L., Correia, A., Diogo, E., Bragança, H., Alves, A., 2016. Diversity

and potential impact of Botryosphaeriaceae species associated with Eucalyptus globulus plantations in Portugal. Eur. J. Plant Pathol. 146, 1–13. doi:10.1007/s10658-016-0910-1
Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. Physiol. Rev. 78, 547–581.
Berger, S., Sinha, A.K., Roitsch, T., 2007. Plant physiology meets phytopathology: plant primary metabolism and plant-pathogen interactions. J. Exp. Bot. 58, 4019–26. doi:10.1093/jxb/erm298
Bettucci, L., Alonso, R., Tiscornia, S., 1999. Endophytic mycobiota of healthy twigs and the assemblage of species associated with twig lesions of Eucalyptus globulus and E. grandis in Uruguay. Mycol. Res. 103, 468–472. doi:10.1017/S0953756298007205
Bilgin, D.D., Zavala, J.A., Zhu, J.I.N., Clough, S.J., Ort, D.R., De Lucia, E., 2010. Biotic stress globally downregulates photosynthesis genes. Plant. Cell Environ. 33, 1597–1613. doi:10.1111/j.1365-3040.2010.02167.x
Blenkinsop, S., Fowler, H., 2007. Changes in European drought characteristics projected by the PRUDENCE regional climate models. Int. J. Climatol. 1610, 1595–1610. doi:http://dx.doi.org/10.1002/joc.1538
Blodgett, J.T., Kruger, E.L., Stanosz, G.R., 1997. Effects of moderate water stress on disease development by Sphaeropsis sapinea on red pine. Phytopathology 87, 422–428.
Blodgett, J.T., Stanosz, G.R., 1997. Sphaeropsis sapinea morphotypes differ in aggressiveness, but both infect nonwounded red or jack pines. Plant Dis. 81, 143–147. doi:10.1094/PDIS.1997.81.2.143
Bogeat-Triboulot, M.-B., Brosché, M., Renaut, J., Jouve, L., Le Thiec, D., Fayyaz, P., Vinocur, B., Witters, E., Laukens, K., Teichmann, T., 2007. Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in Populus euphratica, a poplar growing in arid regions. Plant Physiol. 143, 876–892.
Boller, T., Felix, G., 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol. 60, 379–406. doi:10.1146/annurev.arplant.57.032905.105346
Bolouri, M.M.R., Van den Ende, W., 2012. Sugars and plant innate immunity. J. Exp. Bot. 63, 3989–3998. doi:10.1093/jxb/ers129
Bolton, M.D., 2009. Primary metabolism and plant defense-fuel for the fire. Mol. Plant-Microbe Interact. 22, 487–497.
Bonfig, K.B., Schreiber, U., Gabler, A., Roitsch, T., Berger, S., 2006. Infection with virulent and avirulent P. syringae strains differentially affects photosynthesis and sink metabolism in Arabidopsis leaves. Planta 225, 1–12. doi:10.1007/s00425-006-0303-3
Booth, T.H., 2013. Eucalypt plantations and climate change. For. Ecol. Manage. 301, 28–34. doi:10.1016/j.foreco.2012.04.004
Borralho, N.M.G., Almeida, M.H., Potts, B.M., 2007. O melhoramento do eucalipto em Portugal, in: O Eucaliptal Em Portugal: Impactes Ambientais E Investigação Científica. Instituto Superior de Agronomia, Lisboa, pp. 61–110.
Bostock, R.M., Pye, M.F., Roubtsova, T. V, 2014. Predisposition in plant disease: exploiting the nexus in abiotic and biotic stress perception and response. Annu. Rev. Phytopathol. 52, 517–549. doi:10.1146/annurev-phyto-081211-172902
Boyer, J.S., 1995. Biochemical and biophysical aspects of water deficits and the predisposition to disease. Annu. Rev. Phytopathol. 33, 251–274. doi:10.1146/annurev.py.33.090195.001343
Boyer, J.S., 1982. Plant productivity and environment. Science (80-.). 218, 443–448.
Bragança, H., Diogo, E.L.F., Neves, L., Valente, C., Araújo, C., Bonifácio, L., Phillips, A.J.L., 2015. Quambalaria eucalypti a pathogen of Eucalyptus globulus newly reported in Portugal and in Europe. For. Pathol. 46, 67–75. doi:10.1111/efp.12221
Brooker, M.I.H., Hopper, S.D., 2002. Taxonomy of species deriving from the publication of Eucalyptus subseries Cornutae (Myrtaceae). Nuytsia 14, 325–360.

Brown-Rytlewski, D.E., McManus, P.S., 2000. Virulence of Botryosphaeria dothidea and Botryosphaeria obtusa on apple and management of stem cankers with fungicides. Plant Dis. 84, 1031–1037. doi:10.1094/PDIS.2000.84.9.1031

Cakmak, I., Horst, W.J., 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). Physiol. Plant. 83, 463–468. doi:10.1111/j.1399-3054.1991.tb00121.x

Cazzonelli, C.I., 2011. Goldacre Review: Carotenoids in nature: insights from plants and beyond. Funct. Plant Biol. 38, 833–847. doi:10.1071/FP11192

CELPA, 2014. Boletim Estatístico - Indústria Papeleira Portuguesa. Lisboa.

Cha-um, S., Kirdmanee, C., 2010. Effects of water stress induced by sodium chloride and mannitol on proline accumulation, photosynthetic abilities and growth characters of eucalyptus (Eucalyptus camaldulensis Dehnh.). New For. 40, 349–360.

Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103, 551–560. doi:10.1093/aob/mcn125

Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Review : Understanding plant responses to drought — from genes to the whole plant 239–264. doi:10.1071/FP02076

Chaves, M.M., Oliveira, M.M., 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. J. Exp. Bot. 55, 2365–2384. doi:10.1093/jxb/erh269

Chelli-Chaabouni, A., 2014. Mechanisms and Adaptation of Plants to Environmental Stress: A Case of Woody Species, in: Physiological Mechanisms and Adaptation Strategies in Plants Under Changing Environment. Springer, pp. 1–24.

Chen, H., Jiang, J.-G., 2010. Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. Environ. Rev. 18, 309–319. doi:10.1139/A10-014

Chen, L.-Q., Hou, B.-H., Lalonde, S., Takanaga, H., Hartung, M.L., Qu, X.-Q., Guo, W.-J., Kim, J.-G., Underwood, W., Chaudhuri, B., 2010. Sugar transporters for intercellular exchange and nutrition of pathogens. Nature 468, 527–532.

Chou, H., Bundock, N., Rolfe, S.A., Scholes, J.D., 2000. Infection of Arabidopsis thaliana leaves with Albugo candida (white blister rust) causes a reprogramming of host metabolism. Mol. Plant Pathol. 1, 99–113.

Choudhary, N.L., Sairam, R.K., Tyagi, A., 2005. Expression of D ^{1}-pyrroline-5-carboxylate synthetase gene during drought in rice (Oryza sativa L.). Indian J. Biochem. Biophys. 42, 366–370.

Correia, B., Pintó-Marijuan, M., Castro, B.B., Brossa, R., López-Carbonell, M., Pinto, G., 2014a. Hormonal dynamics during recovery from drought in two *Eucalyptus globulus* genotypes: from root to leaf. Plant Physiol. Biochem. 82, 151–160. doi:10.1016/j.plaphy.2014.05.016

Correia, B., Pintó-Marijuan, M., Neves, L., Brossa, R., Dias, M.C., Costa, A., Castro, B.B., Araújo, C., Santos, C., Chaves, M.M., Pinto, G., 2014b. Water stress and recovery in the performance of two *Eucalyptus globulus* clones: physiological and biochemical profiles. Physiol. Plant. 150, 580–92. doi:10.1111/ppl.12110

Correia, B., Valledor, L., Hancock, R., Jesus, C., Amaral, J., Meijon, M., Pinto, G., 2016. Depicting how *Eucalyptus globulus* survives drought: involvement of redox and DNA methylation events. Funct. Plant Biol. 43, 838–850. doi:10.1071/FP16064

Costa, E.S.F., Shvaleva, A., Maroco, J.P., Almeida, M.H., Chaves, M.M., Pereira, J.S., 2004. Responses to water stress in two Eucalyptus globulus clones differing in drought tolerance. Tree Physiol. 24, 1165–1172. doi:10.1093/treephys/24.10.1165

Couée, I., Sulmon, C., Gouesbet, G., El Amrani, A., 2006. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J. Exp. Bot. 57, 449–459. doi:10.1093/jxb/erj027

Crous, P.W., Slippers, B., Wingfield, M.J., Rheeder, J., Marasas, W.F.O., Philips, A.J.L., Alves, A., Burgess, T., Barber, P., Groenewald, J.Z., 2006. Phylogenetic lineages in the

Botryosphaeriaceae. Stud. Mycol. 55, 235–53. doi:10.3114/sim0020
Cunningham Jr, F.X., Gantt, E., 1998. Genes and enzymes of carotenoid biosynthesis in plants. Annu. Rev. Plant Biol. 49, 557–583. doi:10.1146/annurev.arplant.49.1.557
Dai, A., Trenberth, K.E., Qian, T., 2004. A global dataset of Palmer Drought Severity Index for 1870-2002: Relationship with soil moisture and effects of surface warming. J. Hydrometeorol. 5, 1117–1130. doi:10.1175/JHM-386.1
Dardick, C., Ronald, P., 2006. Plant and animal pathogen recognition receptors signal through non-RD kinases. PLOS Pathog. 2, 14–28. doi:10.1371/journal.ppat.0020002
Denancé, N., Sánchez-Vallet, A., Goffner, D., Molina, A., 2013. Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. Front. Plant Sci. 4, 3389. doi:10.3389/fpls.2013.00155
Desprez-Loustau, M.-L., Marçais, B., Nageleisen, L.-M., Piou, D., Vannini, A., 2006. Interactive effects of drought and pathogens in forest trees. Ann. For. Sci. 63, 597–612. doi:10.1051/forest:2006040
Desprez-Loustau, M.-L., Robin, C., Reynaud, G., Déqué, M., Badeau, V., Piou, D., Husson, C., Marçais, B., 2007. Simulating the effects of a climate-change scenario on the geographical range and activity of forest-pathogenic fungi. Can. J. Plant Pathol. 29, 101–120. doi:10.1080/07060660709507447
Ditmarová, Ľ., Kurjak, D., Palmroth, S., Kmeť, J., Střelcová, K., 2009. Physiological responses of Norway spruce (Picea abies) seedlings to drought stress. Tree Physiol. 30, 205–213. doi:10.1093/treephys/tpp116
Dodds, P.N., Rathjen, J.P., 2010. Plant immunity: towards an integrated view of plant–pathogen interactions. Nat. Rev. Genet. 11, 539–548. doi:10.1038/nrg2812
Durgbanshi, A., Arbona, V., Pozo, O., Miersch, O., Sancho, J. V, Gómez-Cadenas, A., 2005. Simultaneous determination of multiple phytohormones in plant extracts by liquid chromatography-electrospray tandem mass spectrometry. J. Agric. Food Chem. 53, 8437–8442.
Enyedi, A.J., Yalpani, N., Silverman, P., Raskin, I., 1992. Localization, conjugation, and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus. Proc. Natl. Acad. Sci. 89, 2480–2484. doi:10.1073/pnas.89.6.2480
Fabro, G., Kovács, I., Pavet, V., Szabados, L., Alvarez, M.E., 2004. Proline accumulation and AtP5CS2 gene activation are induced by plant-pathogen incompatible interactions in Arabidopsis. Mol. Plant-Microbe Interact. 17, 343–350.
FAO, 2015. Global Forest Resources Assessment 2015, Desk Reference. Rome.
Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T.D., 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biol. 6, 269–279.
Florence, R.., 2004. Ecology and Silviculture of Eucalypt Forests, 1st ed. Csiro Publishing.
Flowers, J., Nuckles, E., Hartman, J., Vaillancourt, L., 2001. Latent infection of Austrian and Scots pine tissues by Sphaeropsis sapinea. Plant Dis. 85, 1107–1112.
Flynn, B., 2010. Eucalyptus: having an impact on the global solid wood industry (JOUR), Wood Resources International.
Fontaine, F., Pinto, C., Vallet, J., Clément, C., Gomes, A.C., Spagnolo, A., 2016. The effects of grapevine trunk diseases (GTDs) on vine physiology. Eur. J. Plant Pathol. 144, 707–721. doi:10.1007/s10658-015-0770-0
Foster, G., Rahmstorf, S., 2011. Global temperature evolution 1979–2010. Environ. Res. Lett. 6, 44022. doi:10.1088/1748-9326/6/4/044022
Franceschini, A., Linaldeddu, B.T., Pisanu, P., Pisanu, S., 2004. Effects of water stress on the endophytic incidence of Biscogniauxia mediterbanea in cork oak trees. J. Plant Pathol. 86, 319–320.
Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., Ryals, J., 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. Science (80-.). 261, 754–756. doi:10.1126/science.261.5122.754

Gao, X., Wang, X., Lu, Y., Zhang, L., Shen, Y., Liang, Z., Zhang, D., 2004. Jasmonic acid is involved in the water stress induced betaine accumulation in pear leaves. Plant. Cell Environ. 27, 497–507. doi:10.1111/j.1365-3040.2004.01167.x

Ghasemi Pirbalouti, A., Sajjadi, S.E., Parang, K., 2014. A Review (Research and Patents) on Jasmonic Acid and Its Derivatives. Arch. Pharm. (Weinheim). 347, 229–239. doi:10.1002/ardp.201300287

Giorgi, F., 2006. Climate change hot-spots. Geophys. Res. Lett. 33.

Glazebrook, J., 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43, 205–227. doi:10.1146/annurev.phyto.43.040204.135923

Gomez-Cadenas, A., Arbona, V., Jacas, J., Primo-Millo, E., Talon, M., 2002. Abscisic acid reduces leaf abscission and increases salt tolerance in citrus plants. J. Plant Growth Regul. 21, 234–240.

Govrin, E.M., Levine, A., 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen Botrytis cinerea. Curr. Biol. 10, 751–757.

Granda, V., Cuesta, C., Álvarez, R., Ordás, R., Centeno, M.L., Rodríguez, A., Majada, J.P., Fernández, B., Feito, I., 2011. Rapid responses of C14 clone of *Eucalyptus globulus* to root drought stress: Time-course of hormonal and physiological signaling. J. Plant Physiol. 168, 661–670. doi:10.1016/j.jplph.2010.09.015

Grulke, N.E., 2011. The nexus of host and pathogen phenology: understanding the disease triangle with climate change. New Phytol. 189, 8–11.

Guarnaschelli, A.B., Prystupa, P., Lemcoff, J.H., 2006. Drought conditioning improves water status, stomatal conductance and survival of Eucalyptus globulus subsp. bicostata seedlings. Ann. For. Sci. 63, 941–950. doi:10.1051/forest:2006077

Halim, V.A., Hunger, A., Macioszek, V., Landgraf, P., Nürnberger, T., Scheel, D., Rosahl, S., 2004. The oligopeptide elicitor Pep-13 induces salicylic acid-dependent and-independent defense reactions in potato. Physiol. Mol. Plant Pathol. 64, 311–318.

Halim, V.A., Vess, A., Scheel, D., Rosahl, S., 2006. The role of salicylic acid and jasmonic acid in pathogen defence. Plant Biol. 8, 307–313. doi:10.1055/s-2006-924025

Haudecoeur, E., Planamente, S., Cirou, A., Tannieres, M., Shelp, B.J., Morera, S., Faure, D., 2009. Proline antagonizes GABA-induced quenching of quorum-sensing in *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. 106, 14587–14592.

Hayat, Q., Hayat, S., Irfan, M., Ahmad, A., 2010. Effect of exogenous salicylic acid under changing environment: a review. Environ. Exp. Bot. 68, 14–25. doi:10.1016/j.envexpbot.2009.08.005

Heller, J., Tudzynski, P., 2011. Reactive oxygen species in phytopathogenic fungi: signaling, development, and disease. Annu. Rev. Phytopathol. 49, 369–390.

Hinchee, M., Rottmann, W., Davis, M., Chang, S., 2011. Eucalyptus with improved pulping and bioenergy characteristics, in: IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY-ANIMAL. SPRINGER 233 SPRING ST, NEW YORK, NY 10013 USA, pp. S29–S30.

Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K., 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207, 604–611. doi:10.1007/s004250050524

Horacio, P., Martinez-Noel, G., 2013. Sucrose signaling in plants: a world yet to be explored. Plant Signal. Behav. 8, 316. doi:10.4161/psb.23316

Horsfall, J.G., Dimond, A.E., 1960. Plant pathology: an advanced treatise. Vol. II: the pathogen. Plant Pathol. an Adv. treatise. Vol. II Pathog.

ICNF, 2013. Instituto de Conservação da Natureza e da Floresta, IFN6 - Áreas dos usos do solo das espécies florestais de Portugal continental. Resultados preliminares. Lisboa.

Iglesias-Trabado, G., Wilstermann, D., 2008. Eucalyptus universalis, Global cultivated eucalypt forests map 2008, Version 1.0. 1.

Irigoyen, J.J., Einerich, D.W., Sánchez-Díaz, M., 1992. Water stress induced changes in

concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiol. Plant. 84, 55–60. doi:10.1111/j.1399-3054.1992.tb08764.x
Ismail, M., Mofizur, R., Hiroshi, H., 2011. Water Stress, Ismail Md. ed. InTech. doi:10.5772/1419
Jactel, H., Petit, J., Desprez-Loustau, M.-L., Delzon, S., Piou, D., Battisti, A., Koricheva, J., 2012. Drought effects on damage by forest insects and pathogens: a meta-analysis. Glob. Chang. Biol. 18, 267–276. doi:10.1111/j.1365-2486.2011.02512.x
Jaleel, C.A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H.J., Somasundaram, R., Panneerselvam, R., 2009. Drought stress in plants: a review on morphological characteristics and pigments composition. Int J Agric Biol 11, 100–105.
Janda, M., Ruelland, E., 2015. Magical mystery tour: salicylic acid signalling. Environ. Exp. Bot. 114, 117–128. doi:10.1016/j.envexpbot.2014.07.003
Jesus, C., Meijón, M., Monteiro, P., Correia, B., Amaral, J., Escandón, M., Cañal, M.J., Pinto, G., 2015. Salicylic acid application modulates physiological and hormonal changes in *Eucalyptus globulus* under water deficit. Environ. Exp. Bot. 118, 56–66. doi:10.1016/j.envexpbot.2015.06.004
Jiang, F., Hartung, W., 2008. Long-distance signalling of abscisic acid (ABA): the factors regulating the intensity of the ABA signal. J. Exp. Bot. 59, 37–43. doi:10.1093/jxb/erm127
Jones, J.D.G., Dangl, J.L., 2006. The plant immune system. Nature 444, 323–329.
Khedr, A.H.A., Abbas, M.A., Wahid, A.A.A., Quick, W.P., Abogadallah, G.M., 2003. Proline induces the expression of salt stress responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt stress. J. Exp. Bot. 54, 2553–2562.
Kissoudis, C., van de Wiel, C., Visser, R.G.F., van der Linden, G., 2014. Enhancing crop resilience to combined abiotic and biotic stress through the dissection of physiological and molecular crosstalk. Front. Plant Sci. 5, 207.
Krause, G.H., Weis, E., 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annu. Rev. Plant Biol. 42, 313–349. doi:10.1146/annurev.pp.42.060191.001525
Kruger, B.M., Manion, P.D., 1994. Antifungal compounds in aspen: effect of water stress. Can. J. Bot. 72, 454–460. doi:10.1139/b94-060
La Porta, N., Capretti, P., Thomsen, I.M., Kasanen, R., Hietala, A.M., Von Weissenberg, K., 2008. Forest pathogens with higher damage potential due to climate change in Europe. Can. J. Plant Pathol. 30, 177–195. doi:10.1080/07060661.2008.10540534
Lamb, C., Dixon, R.A., 1997. The oxidative burst in plant disease resistance. Annu. Rev. Plant Biol. 48, 251–275. doi:10.1146/annurev.arplant.48.1.251
Lawlor, D.W., 2009. Musings about the effects of environment on photosynthesis. Ann. Bot. 103, 543–549.
Lay, F.T., Anderson, M.A., 2005. Defensins - components of the innate immune system in plants. Curr. Protein Pept. Sci. 6, 85–101. doi:10.2174/1389203053027575
Li, C., Berninger, F., Koskela, J., Sonninen, E., 2000. Drought responses of Eucalyptus microtheca provenances depend on seasonality of rainfall in their place of origin. Funct. Plant Biol. 27, 231–238. doi:10.1071/PP99056
Li, L., Liu, X., 2004. Effect of Soil Water Stress on the Growth and Eco-physiological Characteristics of Eucalyptus Seedling. JOURNAL-NORTHWEST For. Univ. 19, 14–17.
Lima, W. de P., Jarvis, P., Rhizopoulou, S., 2003. Stomatal responses of Eucalyptus species to elevated CO2 concentration and drought stress. Sci. Agric. 60, 231–238. doi:10.1590/S0103-90162003000200005
Luger, E., 2003. Eucalypt Introduction As Energy Crop.
Luna, E., Pastor, V., Robert, J., Flors, V., Mauch-Mani, B., Ton, J., 2011. Callose deposition: a multifaceted plant defense response. Mol. Plant-Microbe Interact. 24, 183–193.
Luque, J., Parladé, J., Pera, J., 2002. Seasonal changes in susceptibility of Quercus suber to Botryosphaeria stevensii and Phytophthora cinnamomi. Plant Pathol. 51, 338–345.
Ma, Z., Morgan, D.P., Michailides, T.J., 2001. Effects of water stress on Botryosphaeria blight of pistachio caused by Botryosphaeria dothidea. Plant Dis. 85, 745–749.

doi:10.1094/PDIS.2001.85.7.745
Martorell, S., Diaz-Espejo, A., Medrano, H., Ball, M.C., Choat, B., 2014. Rapid hydraulic recovery in Eucalyptus pauciflora after drought: linkages between stem hydraulics and leaf gas exchange. Plant. Cell Environ. 37, 617–626. doi:10.1111/pce.12182
Matysik, J., Bhalu, B., Mohanty, P., 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Curr. Sci. 82, 525–532.
Mauch-Mani, B., Mauch, F., 2005. The role of abscisic acid in plant–pathogen interactions. Curr. Opin. Plant Biol. 8, 409–414. doi:10.1016/j.pbi.2005.05.015
Mayek-Pérez, N., García-Espinosa, R., López-Castañeda, C., Acosta-Gallegos, J.A., Simpson, J., 2002. Water relations, histopathology and growth of common bean (Phaseolus vulgaris L.) during pathogenesis of Macrophomina phaseolina under drought stress. Physiol. Mol. Plant Pathol. 60, 185–195.
Mcintyre, G.A., Jacobi, W.R., Ramaley, A.W., 1996. Factors affecting Cytospora canker occurrence on aspen. J. Arboric. 22, 229.
McKiernan, A.B., Hovenden, M.J., Brodribb, T.J., Potts, B.M., Davies, N.W., O'Reilly-Wapstra, J.M., 2014. Effect of limited water availability on foliar plant secondary metabolites of two *Eucalyptus* species. Environ. Exp. Bot. 105, 55–64. doi:10.1016/j.envexpbot.2014.04.008
McPartland, J.M., 1984. Hyphal morphology of *Botryosphaeria dothidea* in vessels of unstressed and drought-stressed stems of *Betula alba*. Phytopathology 74, 358–362.
Merchant, A., Tausz, M., Arndt, S.K., Adams, M.A., 2006. Cyclitols and carbohydrates in leaves and roots of 13 Eucalyptus species suggest contrasting physiological responses to water deficit. Plant. Cell Environ. 29, 2017–2029. doi:10.1111/j.1365-3040.2006.01577.x
Michailides, T.J., 1991. Pathogenicity, distribution, sources of inoculum, and infection courts of *Botryosphaeria dothidea* on pistachio. Phytopathology 81, 566–573. doi:10.1094/Phyto-81-566
Michelozzi, M., Johnson, J.D., Warrag, E.I., 1995. Response of ethylene and chlorophyll in two eucalyptus clones during drought. New For. 9, 197–204. doi:10.1007/BF00035487
Miller, G., Shulaev, V., Mittler, R., 2008. Reactive oxygen signaling and abiotic stress. Physiol. Plant. 133, 481–489.
Mohali, S., Slippers, B., Wingfield, M.J., 2007. Identification of *Botryosphaeriaceae* from *Eucalyptus*, *Acacia* and *Pinus* in Venezuela. Fungal Divers 25, 103–125.
Moore, G.A., 1987. The variation of bark/wood bond strength with moisture content of P. radiata and three eucalypt species during storage. Aust. For. Res. 17, 73–78.
Morgan, J.M., 1984. Osmoregulation and water stress in higher plants. Annu. Rev. Plant Physiol. 35, 299–319. doi:10.1146/annurev.pp.35.060184.001503
Morkunas, I., Ratajczak, L., 2014. The role of sugar signaling in plant defense responses against fungal pathogens. Acta Physiol. Plant. 36, 1607–1619. doi:10.1007/s11738-014-1559-z
Munné-Bosch, S., Peñuelas, J., 2003. Photo- and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown Phillyrea angustifolia plants. Planta 217, 758–766. doi:10.1007/s00425-003-1037-0
Naidoo, R., Za, S., 2013. The identification and differential expression of Eucalyptus grandis pathogenesis-related genes in response to salicylic acid and methyl jasmonate. Front. Plant Sci. 4, 43–43. doi:10.3389/fpls.2013.00043
Naidoo, S., Külheim, C., Zwart, L., Mangwanda, R., Oates, C.N., Visser, E.A., Wilken, F.E., Mamni, T.B., Myburg, A.A., 2014. Uncovering the defence responses of Eucalyptus to pests and pathogens in the genomics age. Tree Physiol. 34, 931–943. doi:10.1093/treephys/tpu075
Naidoo, S., Naidoo, R., Oates, C., Wilken, F., Myburg, A., 2011. Investigating Eucalyptus–pathogen and pest interactions to dissect broad spectrum defense mechanisms. BMC Proc. 5, 1. doi:10.1186/1753-6561-5-S7-P97
Navarrete-Campos, D., Bravo, L.A., Rubilar, R.A., Emhart, V., Sanhueza, R., 2013. Drought effects on water use efficiency, freezing tolerance and survival of *Eucalyptus globulus*

and *Eucalyptus globulus* × *nitens* cuttings. New For. 44, 119–134. doi:10.1007/s11056-012-9305-0

Niinemets, Ü., 2010. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. For. Ecol. Manage. 260, 1623–1639. doi:10.1016/j.foreco.2010.07.054

Noss, R.F., 2001. Beyond Kyoto: forest management in a time of rapid climate change. Conserv. Biol. 15, 578–590.

Oghenekaro, A.O., Jaber, E., Raffaello, T., Asiegbu, F.O., 2013. Antimicrobial Defenses and Resistance in Forest Trees: Challenges and Perspectives in a Genomic Era. Annu. Rev. Phytopathol. 51, 221–244. doi:10.1146/annurev-phyto-082712-102307

Old, K.M., Gibbs, R., Craig, I., Myers, B.J., Yuan, Z.Q., 1990. Effect of drought and defoliation on the susceptibility of eucalypts to cankers caused by Endothia gyrosa and Botryosphaeria ribis. Aust. J. Bot. 38, 571–581.

Oliveira Fernandes, I., 2015. Infection mechanism of Diplodia corticola.

Osakabe, Y., Kawaoka, A., Nishikubo, N., Osakabe, K., 2012. Responses to environmental stresses in woody plants: key to survive and longevity. J. Plant Res. 125, 1–10. doi:http://dx.doi.org/10.1007/s10265-011-0446-6

Pachauri, R.K., Allen, M.R., Barros, V.R., Broome, J., Cramer, W., Christ, R., Church, J. a., Clarke, L., Dahe, Q., Dasgupta, P., Dubash, N.K., Edenhofer, O., Elgizouli, I., Field, C.B., Forster, P., Friedlingstein, P., Fuglestvedt, J., Gomez-Echeverri, L., Hallegatte, S., Hegerl, G., Howden, M., Jiang, K., Cisneros, B.J., Kattsov, V., Lee, H., Mach, K.J., Marotzke, J., Mastrandrea, M.D., Meyer, L., Minx, J., Mulugetta, Y., O'Brien, K., Oppenheimer, M., Pereira, J.J., Pichs-Madruga, R., Plattner, G.-K., Pörtner, H.-O., Power, S.B., Preston, B., Ravindranath, N.H., Reisinger, A., Riahi, K., Rusticucci, M., Scholes, R., Seyboth, K., Sokona, Y., Stavins, R., Stocker, T.F., Tschakert, P., Vuuren, D. Van, Ypersele, J.-P. Van, 2014. IPCC, 2014: Climate Change 2014: Synthesis Report. Geneva, Switzerland.

Pavlic, D., Slippers, B., Coutinho, T.A., Wingfield, M.J., 2009. Molecular and phenotypic characterization of three phylogenetic species discovered within the Neofusicoccum parvum/N. ribis complex. Mycologia 101, 636–647. doi:10.3852/08-193

Pavlic, D., Slippers, B., Coutinho, T.A., Wingfield, M.J., 2007. Botryosphaeriaceae occurring on native Syzygium cordatum in South Africa and their potential threat to Eucalyptus. Plant Pathol. 56, 624–636. doi:10.1111/j.1365-3059.2007.01608.x

Peleg, Z., Blumwald, E., 2011. Hormone balance and abiotic stress tolerance in crop plants. Curr. Opin. Plant Biol. 14, 290–295. doi:10.1016/j.pbi.2011.02.001

Pérez, C.A., Wingfield, M.J., Slippers, B., Altier, N.A., Blanchette, R.A., 2010. Endophytic and canker-associated Botryosphaeriaceae occurring on non-native Eucalyptus and native Myrtaceae trees in Uruguay. Fungal Divers. 41, 53–69. doi:10.1007/s13225-009-0014-8

Pillay, K., Slippers, B., Wingfield, M.J., Gryzenhout, M., 2013. Diversity and distribution of co-infecting Botryosphaeriaceae from Eucalyptus grandis and Syzygium cordatum in South Africa. South African J. Bot. 84, 38–43. doi:10.1016/j.sajb.2012.09.003

Pinheiro, C., Chaves, M.M., 2011. Photosynthesis and drought: can we make metabolic connections from available data? J. Exp. Bot. 62, 869–882. doi:10.1093/jxb/erq340

Pita, P., Gascó, A., Pardos, J.A., 2003. Xylem cavitation, leaf growth and leaf water potential in *Eucalyptus globulus* clones under well-watered and drought conditions. Funct. plant Biol. 30, 891–899. doi:10.1071/FP03055

Pita, P., Pardos, J.A., 2001. Growth, leaf morphology, water use and tissue water relations of Eucalyptus globulus clones in response to water deficit. Tree Physiol. 21, 599–607. doi:10.1093/treephys/21.9.599

Pozo, M.J., Van Der Ent, S., Van Loon, L.C., Pieterse, C.M.J., 2008. Transcription factor MYC2 is involved in priming for enhanced defense during rhizobacteria-induced systemic resistance in Arabidopsis thaliana. New Phytol. 180, 511–523. doi:10.1111/j.1469-8137.2008.02578.x

Quinn, G.P., Keough, M.J., 2002. Experimental design and data analysis for biologists. Cambridge University Press.
Reddy, A.R., Chaitanya, K.V., Vivekanandan, M., 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol. 161, 1189–1202. doi:10.1016/j.jplph.2004.01.013
Rejeb, I. Ben, Pastor, V., Mauch-Mani, B., 2014. Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. Plants 3, 458–475. doi:10.3390/plants3040458
Roitsch, T., González, M.-C., 2004. Function and regulation of plant invertases: sweet sensations. Trends Plant Sci. 9, 606–613.
Rojas, C.M., Senthil-Kumar, M., Tzin, V., Mysore, K.S., 2014. Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. Front. Plant Sci. 5, 10–3389. doi:10.3389/fpls.2014.00017
Rolland, F., Baena-Gonzalez, E., Sheen, J., 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu. Rev. Plant Biol. 57, 675–709. doi:10.1146/annurev.arplant.57.032905.105441
Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J.A., Hilal, M., Prado, F.E., 2009. Soluble sugars-metabolism, sensing and abiotic stress. Plant Signal Behav 4, 388–393.
Sakalidis, M.L., Hardy, G.E.S.J., Burgess, T.I., 2011. Use of the Genealogical Sorting Index (GSI) to delineate species boundaries in the Neofusicoccum parvum–Neofusicoccum ribis species complex. Mol. Phylogenet. Evol. 60, 333–344. doi:10.1016/j.ympev.2011.04.026
Sakalidis, M.L., Slippers, B., Wingfield, B.D., Hardy, G.E.S.J., Burgess, T.I., 2013. The challenge of understanding the origin, pathways and extent of fungal invasions: global populations of the Neofusicoccum parvum–N. ribis species complex. Divers. Distrib. 19, 873–883. doi:10.1111/ddi.12030
Sánchez-Vallet, A., López, G., Ramos, B., Delgado-Cerezo, M., Riviere, M.-P., Llorente, F., Fernández, P.V., Miedes, E., Estevez, J.M., Grant, M., 2012. Disruption of abscisic acid signaling constitutively activates *Arabidopsis* resistance to the necrotrophic fungus *Plectosphaerella cucumerina*. Plant Physiol. 160, 2109–2124.
Santakumari, M., Berkowitz, G., 1991. Chloroplast volume: cell water potential relationships and acclimation of photosynthesis to leaf water deficits. Photosynth. Res. 28, 9–20. doi:10.1007/BF00027172
Scharte, J., Schon, H., Weis, E., 2005. Photosynthesis and carbohydrate metabolism in tobacco leaves during an incompatible interaction with Phytophthora nicotianae. Plant. Cell Environ. 28, 1421–1435.
Schoeneweiss, D.F., 1978. Water stress as a predisposing factor in plant disease, Water deficits and plant growth. Illinois Natural History Survey, Illinois. doi:10.1016/B978-0-12-424155-8.50008-0
Schreiber, U., 2004. Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview, in: Chlorophyll a Fluorescence. Springer, pp. 279–319.
Shao, H.-B., Chu, L.-Y., Jaleel, C.A., Manivannan, P., Panneerselvam, R., Shao, M.-A., 2009. Understanding water deficit stress-induced changes in the basic metabolism of higher plants–biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. Crit. Rev. Biotechnol. 29, 131–151. doi:10.1080/07388550902869792
Shao, H.-B., Chu, L.-Y., Jaleel, C.A., Zhao, C.-X., 2008. Water-deficit stress-induced anatomical changes in higher plants. C. R. Biol. 331, 215–25. doi:10.1016/j.crvi.2008.01.002
Shvaleva, A.L., Silva, F.C.E., Breia, E., Jouve, L., Hausman, J.F., Almeida, M.H., Maroco, J.P., Rodrigues, M.L., Pereira, J.S., Chaves, M.M., 2006. Metabolic responses to water deficit in two Eucalyptus globulus clones with contrasting drought sensitivity. Tree Physiol. 26, 239–248. doi:10.1093/treephys/26.2.239
Sims, D.A., Gamon, J.A., 2002. Relationships between leaf pigment content and spectral

reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens. Environ. 81, 337–354. doi:10.1016/S0034-4257(02)00010-X
Slippers, B., Burgess, T., Pavlic, D., Ahumada, R., Maleme, H., Mohali, S., Rodas, C., Wingfield, M.J., 2009. A diverse assemblage of Botryosphaeriaceae infect Eucalyptus in native and non-native environments. South. For. a J. For. Sci. 71, 101–110. doi:10.2989/SF.2009.71.2.3.818
Slippers, B., Fourie, G., Crous, P.W., Coutinho, T.A., Wingfield, B.D., Carnegie, A.J., Wingfield, M.J., 2004. Speciation and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees in Australia and South Africa. Stud. Mycol. 50, 343–358.
Slippers, B., Wingfield, M.J., 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. Fungal Biol. Rev. 21, 90–106. doi:10.1016/j.fbr.2007.06.002
Slippers, B., Wingfield, M.J., Gryzenhout, M., 2014. Botryosphaeriaceae species overlap on four unrelated, native South African hosts. Fungal Biol. 118, 168–179. doi:Botryosphaeriaceae species overlap on four unrelated, native South African hosts
Smirnoff, N., Cumbes, Q.J., 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28, 1057–1060. doi:10.1016/0031-9422(89)80182-7
Smith, H., Kemp, G.H.J., Wingfield, M.J., 1994. Canker and die-back of Eucalyptus in South Africa caused by Botryosphaeria dothidea. Plant Pathol. 43, 1031–1034.
Souza, G.M., Cardoso, V.J.M., 2003. Toward a hierarchical concept of plant stress. Isr. J. plant Sci. 51, 29–37. doi:10.1560/P6TM-RJEL-R5FU-Q039
Sperdouli, I., Moustakas, M., 2012. Interaction of proline, sugars, and anthocyanins during photosynthetic acclimation of Arabidopsis thaliana to drought stress. J. Plant Physiol. 169, 577–585. doi:10.1016/j.jplph.2011.12.015
Stanosz, G.R., Smith, D.R., Albers, J.S., 2005. Surveys for asymptomatic persistence of Sphaeropsis sapinea on or in stems of red pine seedlings from seven Great Lakes region nurseries. For. Pathol. 35, 233–244. doi:10.1111/j.1439-0329.2005.00407.x
Stella, J.C., Riddle, J., Piégay, H., Gagnage, M., Trémélo, M.-L., 2013. Climate and local geomorphic interactions drive patterns of riparian forest decline along a Mediterranean Basin river. Geomorphology 202, 101–114. doi:http://dx.doi.org/10.1016/j.geomorph.2013.01.013
Sturrock, R.N., Frankel, S.J., Brown, A. V, Hennon, P.E., Kliejunas, J.T., Lewis, K.J., Worrall, J.J., Woods, A.J., 2011. Climate change and forest diseases. Plant Pathol. 60, 133–149.
Susiluoto, S., Berninger, F., 2007. Interactions between Morphological and Physiological Drought Responses in Eucalyptus microtheca. Silva Fenn. 41, 221–233. doi:10.14214/sf.292
Swarbrick, P.J., Schulzeilefert, P., Scholes, J.D., 2006. Metabolic consequences of susceptibility and resistance (race specific and broad spectrum) in barley leaves challenged with powdery mildew. Plant. Cell Environ. 29, 1061–1076. doi:10.1111/j.1365-3040.2005.01472.x
Swart, W.J., Wingfield, M.J., 1991. Biology and Control of Sphaeropsis sapinea on Pinus Species in South Africa. Plant Dis. 30, 40.
Szabados, L., Savoure, A., 2010. Proline: a multifunctional amino acid. Trends Plant Sci. 15, 89–97.
Teale, W.D., Paponov, I.A., Palme, K., 2006. Auxin in action: signalling, transport and the control of plant growth and development. Nat. Rev. Mol. Cell Biol. 7, 847–859. doi:10.1038/nrm2020
ter Braak, C.J.F., Verdonschot, P.F.M., 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquat. Sci. 57, 255–289.
Thuiller, W., Lavorel, S., Araújo, M.B., Sykes, M.T., Prentice, I.C., 2005. Climate change threats to plant diversity in Europe. Proc. Natl. Acad. Sci. U. S. A. 102, 8245–8250. doi:10.1073/pnas.0409902102
Trouvelot, S., Héloir, M.-C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., Combier, M., Tdra,

L., Daire, X., Adrian, M., 2014. Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. Front. Plant Sci. 5, 1–14. doi:10.3389/fpls.2014.00592
Trumbore, S., Brando, P., Hartmann, H., 2015. Forest health and global change. Science (80-.). 349, 814–818. doi:10.1126/science.aac6759
Turnbull, J.W., 1999. Eucalypt plantations. New For. 17, 37–52. doi:10.1023/A:1006524911242
Van Niekerk, J., Strever, A.E., Du Toit, G.P., Halleen, F., Fourie, P.H., 2011. Influence of water stress on Botryosphaeriaceae disease expression in grapevines. Phytopathol. Mediterr. 50, 151–165. doi:10.14601/Phytopathol_Mediterr-8968
Villar, E., Klopp, C., Noirot, C., Novaes, E., Kirst, M., Plomion, C., Gion, J.-M., 2011. RNA-Seq reveals genotype-specific molecular responses to water deficit in eucalyptus. BMC Genomics 12, 538. doi:10.1186/1471-2164-12-538
Walley, J.W., Coughlan, S., Hudson, M.E., Covington, M.F., Kaspi, R., Banu, G., Harmer, S.L., Dehesh, K., 2007. Mechanical stress induces biotic and abiotic stress responses via a novel cis-element. PLoS Genet 3, e172.
Wang, D., Pajerowska-Mukhtar, K., Culler, A.H., Dong, X., 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Curr. Biol. 17, 1784–1790. doi:10.1016/j.cub.2007.09.025
Wang, S.Y., 1999. Methyl jasmonate reduces water stress in strawberry. J. Plant Growth Regul. 18, 127–134. doi:10.1007/PL00007060
Warren, C.R., Aranda, I., Cano, F.J., 2011. Responses to water stress of gas exchange and metabolites in Eucalyptus and Acacia spp. Plant. Cell Environ. 34, 1609–29. doi:10.1111/j.1365-3040.2011.02357.x
White, D.A., Beadle, C.L., Worledge, D., 1996a. Leaf water relations of *Eucalyptus globulus* ssp. *globulus* and *E. nitens*: seasonal, drought and species effects. Tree Physiol. 16, 469–476.
White, D.A., Beadle, C.L., Worledge, D., 1996b. Leaf water relations of Eucalyptus globulus ssp. globulus and E. nitens: seasonal, drought and species effects. Tree Physiol. 16, 469–76. doi:10.1093/treephys/16.5.469
Wilhelm, C., Selmar, D., 2011. Energy dissipation is an essential mechanism to sustain the viability of plants: the physiological limits of improved photosynthesis. J. Plant Physiol. 168, 79–87. doi:10.1016/j.jplph.2010.07.012
Wright, A.F., Harmon, P.F., 2010. Identification of species in the Botryosphaeriaceae family causing stem blight on southern highbush blueberry in Florida. Plant Dis. 94, 966–971. doi:10.1094/PDIS-94-8-0966
Xu, Z., Zhou, G., Shimizu, H., 2010. Plant responses to drought and rewatering. Plant Signal. Behav. 5, 649–54. doi:10.4161/psb.5.6.11398
Zhou, S.-X., Medlyn, B.E., Prentice, I.C., 2016. Long-term water stress leads to acclimation of drought sensitivity of photosynthetic capacity in xeric but not riparian Eucalyptus species. Ann. Bot. 117, 133–144. doi:10.1093/aob/mcv161

Part III
Final Considerations

Drought-disease interactions are of particular relevance to forest trees. Drought-induced diseases are frequently caused by latent endophytic pathogens that hold the ability to be present on the plant's tissue and develop disease only when the host is subjected to stress. This way, typically benign organisms become pathogenic, and water stress becomes a revealer of the pathogen's presence. This interaction and predisposition effect represents a major threat to productive and economically important species. This study is included in that framework and was born from the need to fill a knowledge gap; that is, the effect of water stress on the predisposition of *E. globulus,* a worldwide established tree, with a pronounced economic value, to *N. kwambonambiense,* an opportunist endophytic highly aggressive phytopathogen. An interaction projected to escalate as a result of a climate-change-driven temperature increase and summer precipitation decrease.

The secondary objectives of this work were related with the assessment of the individual effects of water-stress and pathogen infection on *E. globulus*. The main objective was to assess the water stress predisposing effect on *E. globulus* and find out if that would result in an increased susceptibility to *N. kwambonambiense* infection and disease development. For that, all plants were firstly subjected to an acclimation period. After that, two water regimes were established, a well-watered and a water-stressed one. Once water-stressed plants achieved 18% of FC, one well-watered and one water-stressed groups were inoculated with *N. kwambonambiense.* At the end of the experiment, several morpho-physiological and biochemical parameters were assessed.

After measuring growth, external lesion extension, water potential, lipid peroxidation, osmolyte and hormone quantification, we were able to conclude that water stress was the main contributor to growth impairment and, therefore, water-stress associated parameters' changes. Well-watered plants (Fig. 1a) presented superior heights, consistent with the IAA content. Despite being infected with *N. kwambonambiense*, presented an overall photosynthetic-parameters' improvement (A, gs, E, ΦPSII). Such allowed plant protection through the production of sugars (TSS), proline and hormones (salicylic acid). Oxidative damages, (partially observed through MDA content), to have happened, were avoided, in part, due to the action of proline and soluble sugars. Such allowed a high-growth maintenance and inferior fungi internal and external progression.

Water-stress (Fig. 1b) decreased water potential, which together with an ABA content increase, lead to stomatal closure. Plants, although experiencing an increase of intercellular

CO_2 concentration, were found unable to use it with a consequent photosynthetic decline (A, E, gs). Such lead to a presumable decrease in the production of enzymes and proteins required in pathogen defense mechanisms and therefore, impairment of the plant's ability to properly react to fungi infection. Oxidative damages (partially observed through MDA content) weren't properly managed and further affected *E. globulus*. A SA content increase was found and, for the first time, under water-stress conditions, *N. kwambonambiense* was found to promote a JA content increase, typical of necrotrophic pathogens. Such may suggest a lifestyle change from biotrophic to necrotrophic as plants cells progressively degenerate.

It is possible to conclude that water stress negatively influenced *E. globulus*, affecting its ability to respond to *N. kwambonambiense* infection. Such is justified by the consistently larger external lesion extensions found in water-stressed, *N. kwambonambiense*-inoculated plants.An increasing number of studies have been revealing the diversity and functional importance of fungal endophytes is trees. This study further represents a clear example of a positive association between drought and disease, i.e., drought or drought and disease acting synergistically affecting tree health. A matter of concern that should be further studied as the effects of climate-change are becoming more severe.

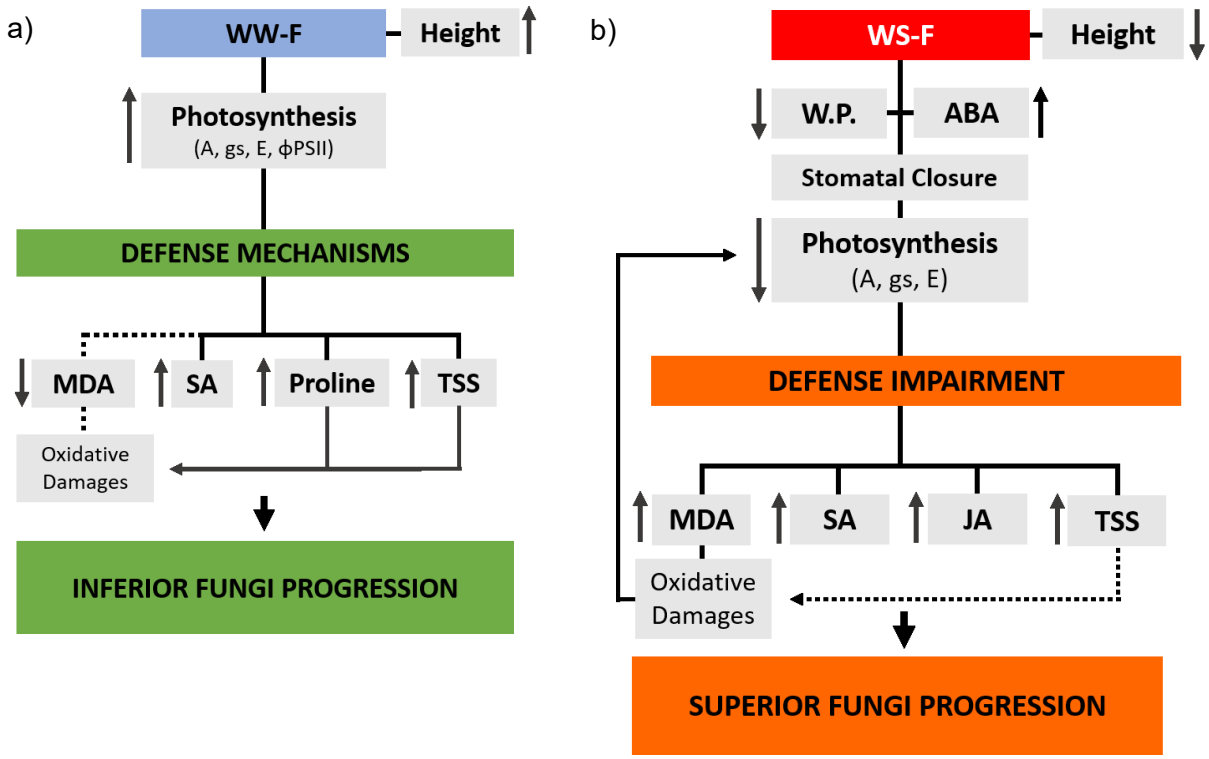

Figure 1 Graphical representation of the obtained results of well-watered and water-stressed *N. kwambonambiense*-inoculated plants

References

Abreo, E., Martinez, S., Bettucci, L., Lupo, S., 2013. Characterization of Botryosphaeriaceae species associated with grapevines in Uruguay. Australas. Plant Pathol. 42, 241–249. doi:10.1007/s13313-013-0200-8

Alvarez, M.E., 2000. Salicylic acid in the machinery of hypersensitive cell death and disease resistance. Plant Mol. Biol. 44, 429–442. doi:10.1023/A:1026561029533

Alves, A., Barradas, C., Phillips, A.J.L., Correia, A., 2013. Diversity of Botryosphaeriaceae species associated with conifers in Portugal. Eur. J. plant Pathol. 135, 791–804.

An, C., Mou, Z., 2011. Salicylic acid and its function in plant immunity. J. Integr. Plant Biol. 53, 412–428. doi:10.1111/j.1744-7909.2011.01043.x

Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R., Kazan, K., 2004. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis*. Plant Cell 16, 3460–3479.

Anjum, S.A., Xie, X., Wang, L.C., Saleem, M.F., Man, C., Lei, W., 2011. Morphological, physiological and biochemical responses of plants to drought stress. African J. Agric. Res. 6, 2026–2032. doi:10.5897/AJAR10.027

Aranjuelo, I., Molero, G., Erice, G., Avice, J.C., Nogués, S., 2011. Plant physiology and proteomics reveals the leaf response to drought in alfalfa (*Medicago sativa* L.). J. Exp. Bot. 62, 111–123. doi:10.1093/jxb/erq249

Bandurska, H., 2005. The effect of salicylic acid on barley response to water deficit. Acta Physiol. Plant. 27, 379–386. doi:10.1007/s11738-005-0015-5

Bari, R., Jones, J.D.G., 2009. Role of plant hormones in plant defence responses. Plant Mol. Biol. 69, 473–488.

Barradas, C., Phillips, A.J.L., Correia, A., Diogo, E., Bragança, H., Alves, A., 2016. Diversity and potential impact of Botryosphaeriaceae species associated with *Eucalyptus globulus* plantations in Portugal. Eur. J. Plant Pathol. 146, 1–13. doi:10.1007/s10658-016-0910-1

Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. Physiol. Rev. 78, 547–581.

Berger, S., Sinha, A.K., Roitsch, T., 2007. Plant physiology meets phytopathology: plant primary metabolism and plant-pathogen interactions. J. Exp. Bot. 58, 4019–26. doi:10.1093/jxb/erm298

Bettucci, L., Alonso, R., Tiscornia, S., 1999. Endophytic mycobiota of healthy twigs and the assemblage of species associated with twig lesions of *Eucalyptus globulus* and *E. grandis* in Uruguay. Mycol. Res. 103, 468–472. doi:10.1017/S0953756298007205

Bilgin, D.D., Zavala, J.A., Zhu, J.I.N., Clough, S.J., Ort, D.R., De Lucia, E., 2010. Biotic stress globally downregulates photosynthesis genes. Plant. Cell Environ. 33, 1597–1613. doi:10.1111/j.1365-3040.2010.02167.x

Blenkinsop, S., Fowler, H., 2007. Changes in European drought characteristics projected by the PRUDENCE regional climate models. Int. J. Climatol. 1610, 1595–1610. doi:http://dx.doi.org/10.1002/joc.1538

Blodgett, J.T., Kruger, E.L., Stanosz, G.R., 1997. Effects of moderate water stress on disease development by *Sphaeropsis sapinea* on red pine. Phytopathology 87, 422–428.

Blodgett, J.T., Stanosz, G.R., 1997. *Sphaeropsis sapinea* morphotypes differ in aggressiveness, but both infect nonwounded red or jack pines. Plant Dis. 81, 143–147. doi:10.1094/PDIS.1997.81.2.143

Bogeat-Triboulot, M.-B., Brosché, M., Renaut, J., Jouve, L., Le Thiec, D., Fayyaz, P., Vinocur, B., Witters, E., Laukens, K., Teichmann, T., 2007. Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. Plant Physiol. 143, 876–892.

Boller, T., Felix, G., 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol. 60, 379–406. doi:10.1146/annurev.arplant.57.032905.105346
Bolouri, M.M.R., Van den Ende, W., 2012. Sugars and plant innate immunity. J. Exp. Bot. 63, 3989–3998. doi:10.1093/jxb/ers129
Bolton, M.D., 2009. Primary metabolism and plant defense-fuel for the fire. Mol. Plant-Microbe Interact. 22, 487–497.
Bonfig, K.B., Schreiber, U., Gabler, A., Roitsch, T., Berger, S., 2006. Infection with virulent and avirulent P. syringae strains differentially affects photosynthesis and sink metabolism in Arabidopsis leaves. Planta 225, 1–12. doi:10.1007/s00425-006-0303-3
Booth, T.H., 2013. Eucalypt plantations and climate change. For. Ecol. Manage. 301, 28–34. doi:10.1016/j.foreco.2012.04.004
Borralho, N.M.G., Almeida, M.H., Potts, B.M., 2007. O melhoramento do eucalipto em Portugal, in: O Eucaliptal Em Portugal: Impactes Ambientais E Investigação Científica. Instituto Superior de Agronomia, Lisboa, pp. 61–110.
Bostock, R.M., Pye, M.F., Roubtsova, T. V, 2014. Predisposition in plant disease: exploiting the nexus in abiotic and biotic stress perception and response. Annu. Rev. Phytopathol. 52, 517–549. doi:10.1146/annurev-phyto-081211-172902
Boyer, J.S., 1995. Biochemical and biophysical aspects of water deficits and the predisposition to disease. Annu. Rev. Phytopathol. 33, 251–274. doi:10.1146/annurev.py.33.090195.001343
Boyer, J.S., 1982. Plant productivity and environment. Science (80-.). 218, 443–448.
Bragança, H., Diogo, E.L.F., Neves, L., Valente, C., Araújo, C., Bonifácio, L., Phillips, A.J.L., 2015. *Quambalaria eucalypti* a pathogen of *Eucalyptus globulus* newly reported in Portugal and in Europe. For. Pathol. 46, 67–75. doi:10.1111/efp.12221
Brooker, M.I.H., Hopper, S.D., 2002. Taxonomy of species deriving from the publication of *Eucalyptus* subseries Cornutae (Myrtaceae). Nuytsia 14, 325–360.
Brown-Rytlewski, D.E., McManus, P.S., 2000. Virulence of *Botryosphaeria dothidea* and Botryosphaeria obtusa on apple and management of stem cankers with fungicides. Plant Dis. 84, 1031–1037. doi:10.1094/PDIS.2000.84.9.1031
Cakmak, I., Horst, W.J., 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiol. Plant. 83, 463–468. doi:10.1111/j.1399-3054.1991.tb00121.x
Cazzonelli, C.I., 2011. Goldacre Review: Carotenoids in nature: insights from plants and beyond. Funct. Plant Biol. 38, 833–847. doi:10.1071/FP11192
CELPA, 2014. Boletim Estatístico - Indústria Papeleira Portuguesa. Lisboa.
Cha-um, S., Kirdmanee, C., 2010. Effects of water stress induced by sodium chloride and mannitol on proline accumulation, photosynthetic abilities and growth characters of eucalyptus (*Eucalyptus camaldulensis* Dehnh.). New For. 40, 349–360.
Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103, 551–560. doi:10.1093/aob/mcn125
Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Review : Understanding plant responses to drought — from genes to the whole plant 239–264. doi:10.1071/FP02076
Chaves, M.M., Oliveira, M.M., 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. J. Exp. Bot. 55, 2365–2384. doi:10.1093/jxb/erh269
Chelli-Chaabouni, A., 2014. Mechanisms and Adaptation of Plants to Environmental Stress: A Case of Woody Species, in: Physiological Mechanisms and Adaptation Strategies in Plants Under Changing Environment. Springer, pp. 1–24.
Chen, H., Jiang, J.-G., 2010. Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. Environ. Rev. 18, 309–319. doi:10.1139/A10-014
Chen, L.-Q., Hou, B.-H., Lalonde, S., Takanaga, H., Hartung, M.L., Qu, X.-Q., Guo, W.-J., Kim,

J.-G., Underwood, W., Chaudhuri, B., 2010. Sugar transporters for intercellular exchange and nutrition of pathogens. Nature 468, 527–532.
Chou, H., Bundock, N., Rolfe, S.A., Scholes, J.D., 2000. Infection of *Arabidopsis thaliana* leaves with Albugo candida (white blister rust) causes a reprogramming of host metabolism. Mol. Plant Pathol. 1, 99–113.
Choudhary, N.L., Sairam, R.K., Tyagi, A., 2005. Expression of D ^{1}-pyrroline-5-carboxylate synthetase gene during drought in rice (*Oryza sativa* L.). Indian J. Biochem. Biophys. 42, 366–370.
Correia, B., Pintó-Marijuan, M., Castro, B.B., Brossa, R., López-Carbonell, M., Pinto, G., 2014a. Hormonal dynamics during recovery from drought in two *Eucalyptus globulus* genotypes: from root to leaf. Plant Physiol. Biochem. 82, 151–160. doi:10.1016/j.plaphy.2014.05.016
Correia, B., Pintó-Marijuan, M., Neves, L., Brossa, R., Dias, M.C., Costa, A., Castro, B.B., Araújo, C., Santos, C., Chaves, M.M., Pinto, G., 2014b. Water stress and recovery in the performance of two *Eucalyptus globulus* clones: physiological and biochemical profiles. Physiol. Plant. 150, 580–92. doi:10.1111/ppl.12110
Correia, B., Valledor, L., Hancock, R., Jesus, C., Amaral, J., Meijon, M., Pinto, G., 2016. Depicting how *Eucalyptus globulus* survives drought: involvement of redox and DNA methylation events. Funct. Plant Biol. 43, 838–850. doi:10.1071/FP16064
Costa, E.S.F., Shvaleva, A., Maroco, J.P., Almeida, M.H., Chaves, M.M., Pereira, J.S., 2004. Responses to water stress in two *Eucalyptus globulus* clones differing in drought tolerance. Tree Physiol. 24, 1165–1172. doi:10.1093/treephys/24.10.1165
Couée, I., Sulmon, C., Gouesbet, G., El Amrani, A., 2006. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J. Exp. Bot. 57, 449–459. doi:10.1093/jxb/erj027
Crous, P.W., Slippers, B., Wingfield, M.J., Rheeder, J., Marasas, W.F.O., Philips, A.J.L., Alves, A., Burgess, T., Barber, P., Groenewald, J.Z., 2006. Phylogenetic lineages in the Botryosphaeriaceae. Stud. Mycol. 55, 235–53. doi:10.3114/sim0020
Cunningham Jr, F.X., Gantt, E., 1998. Genes and enzymes of carotenoid biosynthesis in plants. Annu. Rev. Plant Biol. 49, 557–583. doi:10.1146/annurev.arplant.49.1.557
Dai, A., Trenberth, K.E., Qian, T., 2004. A global dataset of Palmer Drought Severity Index for 1870-2002: Relationship with soil moisture and effects of surface warming. J. Hydrometeorol. 5, 1117–1130. doi:10.1175/JHM-386.1
Dardick, C., Ronald, P., 2006. Plant and animal pathogen recognition receptors signal through non-RD kinases. PLOS Pathog. 2, 14–28. doi:10.1371/journal.ppat.0020002
Denancé, N., Sánchez-Vallet, A., Goffner, D., Molina, A., 2013. Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. Front. Plant Sci. 4, 3389. doi:10.3389/fpls.2013.00155
Desprez-Loustau, M.-L., Marçais, B., Nageleisen, L.-M., Piou, D., Vannini, A., 2006. Interactive effects of drought and pathogens in forest trees. Ann. For. Sci. 63, 597–612. doi:10.1051/forest:2006040
Desprez-Loustau, M.-L., Robin, C., Reynaud, G., Déqué, M., Badeau, V., Piou, D., Husson, C., Marçais, B., 2007. Simulating the effects of a climate-change scenario on the geographical range and activity of forest-pathogenic fungi. Can. J. Plant Pathol. 29, 101–120. doi:10.1080/07060660709507447
Ditmarová, Ľ., Kurjak, D., Palmroth, S., Kmeť, J., Střelcová, K., 2009. Physiological responses of Norway spruce (*Picea abies*) seedlings to drought stress. Tree Physiol. 30, 205–213. doi:10.1093/treephys/tpp116
Dodds, P.N., Rathjen, J.P., 2010. Plant immunity: towards an integrated view of plant–pathogen interactions. Nat. Rev. Genet. 11, 539–548. doi:10.1038/nrg2812
Durgbanshi, A., Arbona, V., Pozo, O., Miersch, O., Sancho, J. V, Gómez-Cadenas, A., 2005. Simultaneous determination of multiple phytohormones in plant extracts by liquid chromatography-electrospray tandem mass spectrometry. J. Agric. Food Chem. 53,

8437–8442.
Enyedi, A.J., Yalpani, N., Silverman, P., Raskin, I., 1992. Localization, conjugation, and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus. Proc. Natl. Acad. Sci. 89, 2480–2484. doi:10.1073/pnas.89.6.2480
Fabro, G., Kovács, I., Pavet, V., Szabados, L., Alvarez, M.E., 2004. Proline accumulation and AtP5CS2 gene activation are induced by plant-pathogen incompatible interactions in *Arabidopsis*. Mol. Plant-Microbe Interact. 17, 343–350.
FAO, 2015. Global Forest Resources Assessment 2015, Desk Reference. Rome.
Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T.D., 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biol. 6, 269–279.
Florence, R.., 2004. Ecology and Silviculture of Eucalypt Forests, 1st ed. Csiro Publishing.
Flowers, J., Nuckles, E., Hartman, J., Vaillancourt, L., 2001. Latent infection of Austrian and Scots pine tissues by Sphaeropsis sapinea. Plant Dis. 85, 1107–1112.
Flynn, B., 2010. Eucalyptus: having an impact on the global solid wood industry (JOUR), Wood Resources International.
Fontaine, F., Pinto, C., Vallet, J., Clément, C., Gomes, A.C., Spagnolo, A., 2016. The effects of grapevine trunk diseases (GTDs) on vine physiology. Eur. J. Plant Pathol. 144, 707–721. doi:10.1007/s10658-015-0770-0
Foster, G., Rahmstorf, S., 2011. Global temperature evolution 1979–2010. Environ. Res. Lett. 6, 44022. doi:10.1088/1748-9326/6/4/044022
Franceschini, A., Linaldeddu, B.T., Pisanu, P., Pisanu, S., 2004. Effects of water stress on the endophytic incidence of *Biscogniauxia mediterbanea* in cork oak trees. J. Plant Pathol. 86, 319–320.
Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., Ryals, J., 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. Science (80-.). 261, 754–756. doi:10.1126/science.261.5122.754
Gao, X., Wang, X., Lu, Y., Zhang, L., Shen, Y., Liang, Z., Zhang, D., 2004. Jasmonic acid is involved in the water stress induced betaine accumulation in pear leaves. Plant. Cell Environ. 27, 497–507. doi:10.1111/j.1365-3040.2004.01167.x
Ghasemi Pirbalouti, A., Sajjadi, S.E., Parang, K., 2014. A Review (Research and Patents) on Jasmonic Acid and Its Derivatives. Arch. Pharm. (Weinheim). 347, 229–239. doi:10.1002/ardp.201300287
Giorgi, F., 2006. Climate change hot-spots. Geophys. Res. Lett. 33.
Glazebrook, J., 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43, 205–227. doi:10.1146/annurev.phyto.43.040204.135923
Gomez-Cadenas, A., Arbona, V., Jacas, J., Primo-Millo, E., Talon, M., 2002. Abscisic acid reduces leaf abscission and increases salt tolerance in citrus plants. J. Plant Growth Regul. 21, 234–240.
Govrin, E.M., Levine, A., 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea.* Curr. Biol. 10, 751–757.
Granda, V., Cuesta, C., Álvarez, R., Ordás, R., Centeno, M.L., Rodríguez, A., Majada, J.P., Fernández, B., Feito, I., 2011. Rapid responses of C14 clone of *Eucalyptus globulus* to root drought stress: Time-course of hormonal and physiological signaling. J. Plant Physiol. 168, 661–670. doi:10.1016/j.jplph.2010.09.015
Grulke, N.E., 2011. The nexus of host and pathogen phenology: understanding the disease triangle with climate change. New Phytol. 189, 8–11.
Guarnaschelli, A.B., Prystupa, P., Lemcoff, J.H., 2006. Drought conditioning improves water status, stomatal conductance and survival of *Eucalyptus globulus* subsp. *bicostata* seedlings. Ann. For. Sci. 63, 941–950. doi:10.1051/forest:2006077
Halim, V.A., Hunger, A., Macioszek, V., Landgraf, P., Nürnberger, T., Scheel, D., Rosahl, S., 2004. The oligopeptide elicitor Pep-13 induces salicylic acid-dependent and-independent

defense reactions in potato. Physiol. Mol. Plant Pathol. 64, 311–318.
Halim, V.A., Vess, A., Scheel, D., Rosahl, S., 2006. The role of salicylic acid and jasmonic acid in pathogen defence. Plant Biol. 8, 307–313. doi:10.1055/s-2006-924025
Haudecoeur, E., Planamente, S., Cirou, A., Tannieres, M., Shelp, B.J., Morera, S., Faure, D., 2009. Proline antagonizes GABA-induced quenching of quorum-sensing in *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. 106, 14587–14592.
Hayat, Q., Hayat, S., Irfan, M., Ahmad, A., 2010. Effect of exogenous salicylic acid under changing environment: a review. Environ. Exp. Bot. 68, 14–25. doi:10.1016/j.envexpbot.2009.08.005
Heller, J., Tudzynski, P., 2011. Reactive oxygen species in phytopathogenic fungi: signaling, development, and disease. Annu. Rev. Phytopathol. 49, 369–390.
Hinchee, M., Rottmann, W., Davis, M., Chang, S., 2011. *Eucalyptus* with improved pulping and bioenergy characteristics, in: IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY-ANIMAL. SPRINGER 233 SPRING ST, NEW YORK, NY 10013 USA, pp. S29–S30.
Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K., 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207, 604–611. doi:10.1007/s004250050524
Horacio, P., Martinez-Noel, G., 2013. Sucrose signaling in plants: a world yet to be explored. Plant Signal. Behav. 8, 316. doi:10.4161/psb.23316
Horsfall, J.G., Dimond, A.E., 1960. Plant pathology: an advanced treatise. Vol. II: the pathogen. Plant Pathol. an Adv. treatise. Vol. II Pathog.
ICNF, 2013. Instituto de Conservação da Natureza e da Floresta, IFN6 - Áreas dos usos do solo das espécies florestais de Portugal continental. Resultados preliminares. Lisboa.
Iglesias-Trabado, G., Wilstermann, D., 2008. Eucalyptus universalis, Global cultivated eucalypt forests map 2008, Version 1.0. 1.
Irigoyen, J.J., Einerich, D.W., Sánchez-Díaz, M., 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiol. Plant. 84, 55–60. doi:10.1111/j.1399-3054.1992.tb08764.x
Ismail, M., Mofizur, R., Hiroshi, H., 2011. Water Stress, Ismail Md. ed. InTech. doi:10.5772/1419
Jactel, H., Petit, J., Desprez-Loustau, M.-L., Delzon, S., Piou, D., Battisti, A., Koricheva, J., 2012. Drought effects on damage by forest insects and pathogens: a meta-analysis. Glob. Chang. Biol. 18, 267–276. doi:10.1111/j.1365-2486.2011.02512.x
Jaleel, C.A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H.J., Somasundaram, R., Panneerselvam, R., 2009. Drought stress in plants: a review on morphological characteristics and pigments composition. Int J Agric Biol 11, 100–105.
Janda, M., Ruelland, E., 2015. Magical mystery tour: salicylic acid signalling. Environ. Exp. Bot. 114, 117–128. doi:10.1016/j.envexpbot.2014.07.003
Jesus, C., Meijón, M., Monteiro, P., Correia, B., Amaral, J., Escandón, M., Cañal, M.J., Pinto, G., 2015. Salicylic acid application modulates physiological and hormonal changes in *Eucalyptus globulus* under water deficit. Environ. Exp. Bot. 118, 56–66. doi:10.1016/j.envexpbot.2015.06.004
Jiang, F., Hartung, W., 2008. Long-distance signalling of abscisic acid (ABA): the factors regulating the intensity of the ABA signal. J. Exp. Bot. 59, 37–43. doi:10.1093/jxb/erm127
Jones, J.D.G., Dangl, J.L., 2006. The plant immune system. Nature 444, 323–329.
Khedr, A.H.A., Abbas, M.A., Wahid, A.A.A., Quick, W.P., Abogadallah, G.M., 2003. Proline induces the expression of salt stress responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt stress. J. Exp. Bot. 54, 2553–2562.
Kissoudis, C., van de Wiel, C., Visser, R.G.F., van der Linden, G., 2014. Enhancing crop resilience to combined abiotic and biotic stress through the dissection of physiological and molecular crosstalk. Front. Plant Sci. 5, 207.
Krause, G.H., Weis, E., 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annu.

Rev. Plant Biol. 42, 313–349. doi:10.1146/annurev.pp.42.060191.001525
Kruger, B.M., Manion, P.D., 1994. Antifungal compounds in aspen: effect of water stress. Can. J. Bot. 72, 454–460. doi:10.1139/b94-060
La Porta, N., Capretti, P., Thomsen, I.M., Kasanen, R., Hietala, A.M., Von Weissenberg, K., 2008. Forest pathogens with higher damage potential due to climate change in Europe. Can. J. Plant Pathol. 30, 177–195. doi:10.1080/07060661.2008.10540534
Lamb, C., Dixon, R.A., 1997. The oxidative burst in plant disease resistance. Annu. Rev. Plant Biol. 48, 251–275. doi:10.1146/annurev.arplant.48.1.251
Lawlor, D.W., 2009. Musings about the effects of environment on photosynthesis. Ann. Bot. 103, 543–549.
Lay, F.T., Anderson, M.A., 2005. Defensins - components of the innate immune system in plants. Curr. Protein Pept. Sci. 6, 85–101. doi:10.2174/1389203053027575
Li, C., Berninger, F., Koskela, J., Sonninen, E., 2000. Drought responses of *Eucalyptus microtheca* provenances depend on seasonality of rainfall in their place of origin. Funct. Plant Biol. 27, 231–238. doi:10.1071/PP99056
Li, L., Liu, X., 2004. Effect of Soil Water Stress on the Growth and Eco-physiological Characteristics of Eucalyptus Seedling. JOURNAL-NORTHWEST For. Univ. 19, 14–17.
Lima, W. de P., Jarvis, P., Rhizopoulou, S., 2003. Stomatal responses of *Eucalyptus* species to elevated CO2 concentration and drought stress. Sci. Agric. 60, 231–238. doi:10.1590/S0103-90162003000200005
Luger, E., 2003. Eucalypt Introduction As Energy Crop.
Luna, E., Pastor, V., Robert, J., Flors, V., Mauch-Mani, B., Ton, J., 2011. Callose deposition: a multifaceted plant defense response. Mol. Plant-Microbe Interact. 24, 183–193.
Luque, J., Parladé, J., Pera, J., 2002. Seasonal changes in susceptibility of *Quercus suber* to *Botryosphaeria stevensii* and *Phytophthora cinnamomi*. Plant Pathol. 51, 338–345.
Ma, Z., Morgan, D.P., Michailides, T.J., 2001. Effects of water stress on Botryosphaeria blight of pistachio caused by B*otryosphaeria dothidea*. Plant Dis. 85, 745–749. doi:10.1094/PDIS.2001.85.7.745
Martorell, S., Diaz-Espejo, A., Medrano, H., Ball, M.C., Choat, B., 2014. Rapid hydraulic recovery in *Eucalyptus pauciflora* after drought: linkages between stem hydraulics and leaf gas exchange. Plant. Cell Environ. 37, 617–626. doi:10.1111/pce.12182
Matysik, J., Bhalu, B., Mohanty, P., 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Curr. Sci. 82, 525–532.
Mauch-Mani, B., Mauch, F., 2005. The role of abscisic acid in plant–pathogen interactions. Curr. Opin. Plant Biol. 8, 409–414. doi:10.1016/j.pbi.2005.05.015
Mayek-Pérez, N., García-Espinosa, R., López-Castañeda, C., Acosta-Gallegos, J.A., Simpson, J., 2002. Water relations, histopathology and growth of common bean (Phaseolus vulgaris L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. Physiol. Mol. Plant Pathol. 60, 185–195.
Mcintyre, G.A., Jacobi, W.R., Ramaley, A.W., 1996. Factors affecting Cytospora canker occurrence on aspen. J. Arboric. 22, 229.
McKiernan, A.B., Hovenden, M.J., Brodribb, T.J., Potts, B.M., Davies, N.W., O'Reilly-Wapstra, J.M., 2014. Effect of limited water availability on foliar plant secondary metabolites of two *Eucalyptus* species. Environ. Exp. Bot. 105, 55–64. doi:10.1016/j.envexpbot.2014.04.008
McPartland, J.M., 1984. Hyphal morphology of *Botryosphaeria dothidea* in vessels of unstressed and drought-stressed stems of *Betula alba*. Phytopathology 74, 358–362.
Merchant, A., Tausz, M., Arndt, S.K., Adams, M.A., 2006. Cyclitols and carbohydrates in leaves and roots of 13 Eucalyptus species suggest contrasting physiological responses to water deficit. Plant. Cell Environ. 29, 2017–2029. doi:10.1111/j.1365-3040.2006.01577.x
Michailides, T.J., 1991. Pathogenicity, distribution, sources of inoculum, and infection courts of *Botryosphaeria dothidea* on pistachio. Phytopathology 81, 566–573. doi:10.1094/Phyto-81-566
Michelozzi, M., Johnson, J.D., Warrag, E.I., 1995. Response of ethylene and chlorophyll in two

eucalyptus clones during drought. New For. 9, 197–204. doi:10.1007/BF00035487
Miller, G., Shulaev, V., Mittler, R., 2008. Reactive oxygen signaling and abiotic stress. Physiol. Plant. 133, 481–489.
Mohali, S., Slippers, B., Wingfield, M.J., 2007. Identification of *Botryosphaeriaceae* from *Eucalyptus*, *Acacia* and *Pinus* in Venezuela. Fungal Divers 25, 103–125.
Moore, G.A., 1987. The variation of bark/wood bond strength with moisture content of P. radiata and three eucalypt species during storage. Aust. For. Res. 17, 73–78.
Morgan, J.M., 1984. Osmoregulation and water stress in higher plants. Annu. Rev. Plant Physiol. 35, 299–319. doi:10.1146/annurev.pp.35.060184.001503
Morkunas, I., Ratajczak, L., 2014. The role of sugar signaling in plant defense responses against fungal pathogens. Acta Physiol. Plant. 36, 1607–1619. doi:10.1007/s11738-014-1559-z
Munné-Bosch, S., Peñuelas, J., 2003. Photo- and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. Planta 217, 758–766. doi:10.1007/s00425-003-1037-0
Naidoo, R., Za, S., 2013. The identification and differential expression of *Eucalyptus grandis* pathogenesis-related genes in response to salicylic acid and methyl jasmonate. Front. Plant Sci. 4, 43–43. doi:10.3389/fpls.2013.00043
Naidoo, S., Külheim, C., Zwart, L., Mangwanda, R., Oates, C.N., Visser, E.A., Wilken, F.E., Mamni, T.B., Myburg, A.A., 2014. Uncovering the defence responses of *Eucalyptus* to pests and pathogens in the genomics age. Tree Physiol. 34, 931–943. doi:10.1093/treephys/tpu075
Naidoo, S., Naidoo, R., Oates, C., Wilken, F., Myburg, A., 2011. Investigating *Eucalyptus*–pathogen and pest interactions to dissect broad spectrum defense mechanisms. BMC Proc. 5, 1. doi:10.1186/1753-6561-5-S7-P97
Navarrete-Campos, D., Bravo, L.A., Rubilar, R.A., Emhart, V., Sanhueza, R., 2013. Drought effects on water use efficiency, freezing tolerance and survival of *Eucalyptus globulus* and *Eucalyptus globulus* × *nitens* cuttings. New For. 44, 119–134. doi:10.1007/s11056-012-9305-0
Niinemets, Ü., 2010. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. For. Ecol. Manage. 260, 1623–1639. doi:10.1016/j.foreco.2010.07.054
Noss, R.F., 2001. Beyond Kyoto: forest management in a time of rapid climate change. Conserv. Biol. 15, 578–590.
Oghenekaro, A.O., Jaber, E., Raffaello, T., Asiegbu, F.O., 2013. Antimicrobial Defenses and Resistance in Forest Trees: Challenges and Perspectives in a Genomic Era. Annu. Rev. Phytopathol. 51, 221–244. doi:10.1146/annurev-phyto-082712-102307
Old, K.M., Gibbs, R., Craig, I., Myers, B.J., Yuan, Z.Q., 1990. Effect of drought and defoliation on the susceptibility of eucalypts to cankers caused by *Endothia gyrosa* and *Botryosphaeria ribis*. Aust. J. Bot. 38, 571–581.
Oliveira Fernandes, I. (2015). Infection mechanism of *Diplodia corticola*. Ghent University. Faculty of Sciences ; Universidade de Aveiro. Departamento de Biologia, Ghent, Belgium ; Aveiro, Portugal.
Osakabe, Y., Kawaoka, A., Nishikubo, N., Osakabe, K., 2012. Responses to environmental stresses in woody plants: key to survive and longevity. J. Plant Res. 125, 1–10. doi:http://dx.doi.org/10.1007/s10265-011-0446-6
Pachauri, R.K., Allen, M.R., Barros, V.R., Broome, J., Cramer, W., Christ, R., Church, J. a., Clarke, L., Dahe, Q., Dasgupta, P., Dubash, N.K., Edenhofer, O., Elgizouli, I., Field, C.B., Forster, P., Friedlingstein, P., Fuglestvedt, J., Gomez-Echeverri, L., Hallegatte, S., Hegerl, G., Howden, M., Jiang, K., Cisneros, B.J., Kattsov, V., Lee, H., Mach, K.J., Marotzke, J., Mastrandrea, M.D., Meyer, L., Minx, J., Mulugetta, Y., O'Brien, K., Oppenheimer, M., Pereira, J.J., Pichs-Madruga, R., Plattner, G.-K., Pörtner, H.-O., Power, S.B., Preston, B., Ravindranath, N.H., Reisinger, A., Riahi, K., Rusticucci, M.,

Scholes, R., Seyboth, K., Sokona, Y., Stavins, R., Stocker, T.F., Tschakert, P., Vuuren, D. Van, Ypersele, J.-P. Van, 2014. IPCC, 2014: Climate Change 2014: Synthesis Report. Geneva, Switzerland.

Pavlic, D., Slippers, B., Coutinho, T.A., Wingfield, M.J., 2009. Molecular and phenotypic characterization of three phylogenetic species discovered within the *Neofusicoccum parvum/N. ribis* complex. Mycologia 101, 636–647. doi:10.3852/08-193

Pavlic, D., Slippers, B., Coutinho, T.A., Wingfield, M.J., 2007. Botryosphaeriaceae occurring on native Syzygium cordatum in South Africa and their potential threat to *Eucalyptus*. Plant Pathol. 56, 624–636. doi:10.1111/j.1365-3059.2007.01608.x

Peleg, Z., Blumwald, E., 2011. Hormone balance and abiotic stress tolerance in crop plants. Curr. Opin. Plant Biol. 14, 290–295. doi:10.1016/j.pbi.2011.02.001

Pérez, C.A., Wingfield, M.J., Slippers, B., Altier, N.A., Blanchette, R.A., 2010. Endophytic and canker-associated Botryosphaeriaceae occurring on non-native *Eucalyptus* and native Myrtaceae trees in Uruguay. Fungal Divers. 41, 53–69. doi:10.1007/s13225-009-0014-8

Pillay, K., Slippers, B., Wingfield, M.J., Gryzenhout, M., 2013. Diversity and distribution of co-infecting Botryosphaeriaceae from *Eucalyptus grandis* and *Syzygium cordatum* in South Africa. South African J. Bot. 84, 38–43. doi:10.1016/j.sajb.2012.09.003

Pinheiro, C., Chaves, M.M., 2011. Photosynthesis and drought: can we make metabolic connections from available data? J. Exp. Bot. 62, 869–882. doi:10.1093/jxb/erq340

Pita, P., Gascó, A., Pardos, J.A., 2003. Xylem cavitation, leaf growth and leaf water potential in *Eucalyptus globulus* clones under well-watered and drought conditions. Funct. plant Biol. 30, 891–899. doi:10.1071/FP03055

Pita, P., Pardos, J.A., 2001. Growth, leaf morphology, water use and tissue water relations of *Eucalyptus globulus* clones in response to water deficit. Tree Physiol. 21, 599–607. doi:10.1093/treephys/21.9.599

Pozo, M.J., Van Der Ent, S., Van Loon, L.C., Pieterse, C.M.J., 2008. Transcription factor MYC2 is involved in priming for enhanced defense during rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. New Phytol. 180, 511–523. doi:10.1111/j.1469-8137.2008.02578.x

Quinn, G.P., Keough, M.J., 2002. Experimental design and data analysis for biologists. Cambridge University Press.

Reddy, A.R., Chaitanya, K.V., Vivekanandan, M., 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol. 161, 1189–1202. doi:10.1016/j.jplph.2004.01.013

Rejeb, I. Ben, Pastor, V., Mauch-Mani, B., 2014. Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. Plants 3, 458–475. doi:10.3390/plants3040458

Roitsch, T., González, M.-C., 2004. Function and regulation of plant invertases: sweet sensations. Trends Plant Sci. 9, 606–613.

Rojas, C.M., Senthil-Kumar, M., Tzin, V., Mysore, K.S., 2014. Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. Front. Plant Sci. 5, 10–3389. doi:10.3389/fpls.2014.00017

Rolland, F., Baena-Gonzalez, E., Sheen, J., 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu. Rev. Plant Biol. 57, 675–709. doi:10.1146/annurev.arplant.57.032905.105441

Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J.A., Hilal, M., Prado, F.E., 2009. Soluble sugars-metabolism, sensing and abiotic stress. Plant Signal Behav 4, 388–393.

Sakalidis, M.L., Hardy, G.E.S.J., Burgess, T.I., 2011. Use of the Genealogical Sorting Index (GSI) to delineate species boundaries in the *Neofusicoccum parvum–Neofusicoccum ribis* species complex. Mol. Phylogenet. Evol. 60, 333–344. doi:10.1016/j.ympev.2011.04.026

Sakalidis, M.L., Slippers, B., Wingfield, B.D., Hardy, G.E.S.J., Burgess, T.I., 2013. The challenge of understanding the origin, pathways and extent of fungal invasions: global populations of the *Neofusicoccum parvum–N. ribis* species complex. Divers. Distrib. 19,

873–883. doi:10.1111/ddi.12030
Sánchez-Vallet, A., López, G., Ramos, B., Delgado-Cerezo, M., Riviere, M.-P., Llorente, F., Fernández, P.V., Miedes, E., Estevez, J.M., Grant, M., 2012. Disruption of abscisic acid signaling constitutively activates *Arabidopsis* resistance to the necrotrophic fungus *Plectosphaerella cucumerina*. Plant Physiol. 160, 2109–2124.
Santakumari, M., Berkowitz, G., 1991. Chloroplast volume: cell water potential relationships and acclimation of photosynthesis to leaf water deficits. Photosynth. Res. 28, 9–20. doi:10.1007/BF00027172
Scharte, J., Schon, H., Weis, E., 2005. Photosynthesis and carbohydrate metabolism in tobacco leaves during an incompatible interaction with *Phytophthora nicotianae*. Plant. Cell Environ. 28, 1421–1435.
Schoeneweiss, D.F., 1978. Water stress as a predisposing factor in plant disease, Water deficits and plant growth. Illinois Natural History Survey, Illinois. doi:10.1016/B978-0-12-424155-8.50008-0
Schreiber, U., 2004. Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview, in: Chlorophyll a Fluorescence. Springer, pp. 279–319.
Shao, H.-B., Chu, L.-Y., Jaleel, C.A., Manivannan, P., Panneerselvam, R., Shao, M.-A., 2009. Understanding water deficit stress-induced changes in the basic metabolism of higher plants–biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. Crit. Rev. Biotechnol. 29, 131–151. doi:10.1080/07388550902869792
Shao, H.-B., Chu, L.-Y., Jaleel, C.A., Zhao, C.-X., 2008. Water-deficit stress-induced anatomical changes in higher plants. C. R. Biol. 331, 215–25. doi:10.1016/j.crvi.2008.01.002
Shvaleva, A.L., Silva, F.C.E., Breia, E., Jouve, L., Hausman, J.F., Almeida, M.H., Maroco, J.P., Rodrigues, M.L., Pereira, J.S., Chaves, M.M., 2006. Metabolic responses to water deficit in two *Eucalyptus globulus* clones with contrasting drought sensitivity. Tree Physiol. 26, 239–248. doi:10.1093/treephys/26.2.239
Sims, D.A., Gamon, J.A., 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens. Environ. 81, 337–354. doi:10.1016/S0034-4257(02)00010-X
Slippers, B., Burgess, T., Pavlic, D., Ahumada, R., Maleme, H., Mohali, S., Rodas, C., Wingfield, M.J., 2009. A diverse assemblage of Botryosphaeriaceae infect *Eucalyptus* in native and non-native environments. South. For. a J. For. Sci. 71, 101–110. doi:10.2989/SF.2009.71.2.3.818
Slippers, B., Fourie, G., Crous, P.W., Coutinho, T.A., Wingfield, B.D., Carnegie, A.J., Wingfield, M.J., 2004. Speciation and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees in Australia and South Africa. Stud. Mycol. 50, 343–358.
Slippers, B., Wingfield, M.J., 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. Fungal Biol. Rev. 21, 90–106. doi:10.1016/j.fbr.2007.06.002
Slippers, B., Wingfield, M.J., Gryzenhout, M., 2014. Botryosphaeriaceae species overlap on four unrelated, native South African hosts. Fungal Biol. 118, 168–179. doi: 10.1016/j.funbio.2013.11.007
Smirnoff, N., Cumbes, Q.J., 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28, 1057–1060. doi:10.1016/0031-9422(89)80182-7
Smith, H., Kemp, G.H.J., Wingfield, M.J., 1994. Canker and die-back of *Eucalyptus* in South Africa caused by *Botryosphaeria dothidea*. Plant Pathol. 43, 1031–1034.
Souza, G.M., Cardoso, V.J.M., 2003. Toward a hierarchical concept of plant stress. Isr. J. plant Sci. 51, 29–37. doi:10.1560/P6TM-RJEL-R5FU-Q039
Sperdouli, I., Moustakas, M., 2012. Interaction of proline, sugars, and anthocyanins during photosynthetic acclimation of *Arabidopsis thaliana* to drought stress. J. Plant Physiol. 169, 577–585. doi:10.1016/j.jplph.2011.12.015

Stanosz, G.R., Smith, D.R., Albers, J.S., 2005. Surveys for asymptomatic persistence of *Sphaeropsis sapinea* on or in stems of red pine seedlings from seven Great Lakes region nurseries. For. Pathol. 35, 233–244. doi:10.1111/j.1439-0329.2005.00407.x

Stella, J.C., Riddle, J., Piégay, H., Gagnage, M., Trémélo, M.-L., 2013. Climate and local geomorphic interactions drive patterns of riparian forest decline along a Mediterranean Basin river. Geomorphology 202, 101–114. doi:http://dx.doi.org/10.1016/j.geomorph.2013.01.013

Sturrock, R.N., Frankel, S.J., Brown, A. V, Hennon, P.E., Kliejunas, J.T., Lewis, K.J., Worrall, J.J., Woods, A.J., 2011. Climate change and forest diseases. Plant Pathol. 60, 133–149.

Susiluoto, S., Berninger, F., 2007. Interactions between Morphological and Physiological Drought Responses in *Eucalyptus microtheca*. Silva Fenn. 41, 221–233. doi:10.14214/sf.292

Swarbrick, P.J., Schulzeilefert, P., Scholes, J.D., 2006. Metabolic consequences of susceptibility and resistance (race specific and broad spectrum) in barley leaves challenged with powdery mildew. Plant. Cell Environ. 29, 1061–1076. doi:10.1111/j.1365-3040.2005.01472.x

Swart, W.J., Wingfield, M.J., 1991. Biology and Control of *Sphaeropsis sapinea* on *Pinus* species in South Africa. Plant Dis. 30, 40.

Szabados, L., Savoure, A., 2010. Proline: a multifunctional amino acid. Trends Plant Sci. 15, 89–97.

Teale, W.D., Paponov, I.A., Palme, K., 2006. Auxin in action: signalling, transport and the control of plant growth and development. Nat. Rev. Mol. Cell Biol. 7, 847–859. doi:10.1038/nrm2020

ter Braak, C.J.F., Verdonschot, P.F.M., 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquat. Sci. 57, 255–289.

Thuiller, W., Lavorel, S., Araújo, M.B., Sykes, M.T., Prentice, I.C., 2005. Climate change threats to plant diversity in Europe. Proc. Natl. Acad. Sci. U. S. A. 102, 8245–8250. doi:10.1073/pnas.0409902102

Trouvelot, S., Héloir, M.-C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., Combier, M., Tdra, L., Daire, X., Adrian, M., 2014. Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. Front. Plant Sci. 5, 1–14. doi:10.3389/fpls.2014.00592

Trumbore, S., Brando, P., Hartmann, H., 2015. Forest health and global change. Science (80-.). 349, 814–818. doi:10.1126/science.aac6759

Turnbull, J.W., 1999. Eucalypt plantations. New For. 17, 37–52. doi:10.1023/A:1006524911242

Van Niekerk, J., Strever, A.E., Du Toit, G.P., Halleen, F., Fourie, P.H., 2011. Influence of water stress on Botryosphaeriaceae disease expression in grapevines. Phytopathol. Mediterr. 50, 151–165. doi:10.14601/Phytopathol_Mediterr-8968

Villar, E., Klopp, C., Noirot, C., Novaes, E., Kirst, M., Plomion, C., Gion, J.-M., 2011. RNA-Seq reveals genotype-specific molecular responses to water deficit in eucalyptus. BMC Genomics 12, 538. doi:10.1186/1471-2164-12-538

Walley, J.W., Coughlan, S., Hudson, M.E., Covington, M.F., Kaspi, R., Banu, G., Harmer, S.L., Dehesh, K., 2007. Mechanical stress induces biotic and abiotic stress responses via a novel cis-element. PLoS Genet 3, e172.

Wang, D., Pajerowska-Mukhtar, K., Culler, A.H., Dong, X., 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Curr. Biol. 17, 1784–1790. doi:10.1016/j.cub.2007.09.025

Wang, S.Y., 1999. Methyl jasmonate reduces water stress in strawberry. J. Plant Growth Regul. 18, 127–134. doi:10.1007/PL00007060

Warren, C.R., Aranda, I., Cano, F.J., 2011. Responses to water stress of gas exchange and metabolites in *Eucalyptus* and *Acacia* spp. Plant. Cell Environ. 34, 1609–29. doi:10.1111/j.1365-3040.2011.02357.x

White, D.A., Beadle, C.L., Worledge, D., 1996a. Leaf water relations of *Eucalyptus globulus* ssp. *globulus* and *E. nitens*: seasonal, drought and species effects. Tree Physiol. 16, 469–476.

White, D.A., Beadle, C.L., Worledge, D., 1996b. Leaf water relations of *Eucalyptus globulus* ssp. globulus and E. nitens: seasonal, drought and species effects. Tree Physiol. 16, 469–76. doi:10.1093/treephys/16.5.469

Wilhelm, C., Selmar, D., 2011. Energy dissipation is an essential mechanism to sustain the viability of plants: the physiological limits of improved photosynthesis. J. Plant Physiol. 168, 79–87. doi:10.1016/j.jplph.2010.07.012

Wright, A.F., Harmon, P.F., 2010. Identification of species in the Botryosphaeriaceae family causing stem blight on southern highbush blueberry in Florida. Plant Dis. 94, 966–971. doi:10.1094/PDIS-94-8-0966

Xu, Z., Zhou, G., Shimizu, H., 2010. Plant responses to drought and rewatering. Plant Signal. Behav. 5, 649–54. doi:10.4161/psb.5.6.11398

Zhou, S.-X., Medlyn, B.E., Prentice, I.C., 2016. Long-term water stress leads to acclimation of drought sensitivity of photosynthetic capacity in xeric but not riparian *Eucalyptus* species. Ann. Bot. 117, 133–144. doi:10.1093/aob/mcv161